Research Article

# Isolation, Characterization and Antibactrial Activity of Ecdysteroid from Roots of *Polycarpaea corymbosa* Lam

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**ABSTRACT**

A new phytoecdysteroid (2- deoxy 20 hydroxy 22 cinnamoyl ecdysone 3, 25 diacetate) was isolated from the roots of *Polycarpaea corymbosa* Lam (family: caryophyllaceae).The powdered roots were extracted with petroleum ether ( 60-80° C), benzene, ethyl acetate and ethyl alcohol in soxhlet apparatus by simultaneous extraction. The highest yield of benzene extract was chosen for the isolation of ecdysteroid. The white solid pure compound was isolated and tested by TLC and respond positively for Liebermann - burchard reaction. Their structure has been established by UV, IR, $^{1}H$, $^{13}C$-NMR DEPT -90, DEPT -135 and EI- MS experiments. All the extracts and isolated compound were screened for anti-bacterial activity against *B.subtilis, P.vulgaris, E.coli, P.aeruginosa* by Kirby-bauer method.

**Keywords:** Caryophyllaceae, *Polycarpaea corymbosa*, Isolation, Ecdysteroid derivative, Antibacterial activity.

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## INTRODUCTION

Plants belonging to the family Caryophyllaceae are frequently used in traditional medicine and have been reported to be rich in ecdysteroid.[1] The *Polycarpaea corymbosa* are commonly used in traditional medicine as a strangury, urinary calculi, boils, inflammatory swelling, ulcers, and snakebite and also used for Jaundice.[2] The different extracts have been studied for the antifungal activity.[3]

Plants of the genus polycarpaea (Fam. Caryophyllaceae) appear promising in the search for ecdysone containing plants among representatives of the domestic flora. The caryophyllaceae family is distinguished from other families in which various ecdysteroids have been detected.[4]

Here we selected the plant *Polycarpaea corymbosa* (Fam. Caryophyllaceae) traditionally used in the treatment of jaundice, snakebite, and inflammation in our area of Sirumallai hills, Dindigul, Tamil Nadu. A perusal of the literature reveals that there are no prior reports with regard to the chemistry of this plant. So, we planned to isolate the steroid, ecdysteroid which is the main constituent of the family caryphyllaceae and to elucidate the structure by chemical, physical and spectral analysis as well as to investigate the antibacterial activity of the plant extracts and isolated compound.

## MATERIALS AND METHODS

### Plant Sources

Polycarpaea croymbosa roots were collected from Sirumalai hills at Dindigul of Tamilnadu and authenticated by Prof. P. Jeyaraman (Plant anatomy research centre, Chennai). A Voucher specimen of the sample was deposited in the Department of Pharmaceutical Analysis for future reference. The plant picture is shown in figure 1 & 2.

**Figure 1:** Plant picture (*Polycarpaea corymbosa*)

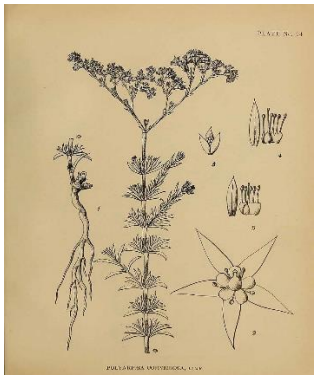


**Figure 2:** Plant picture in Indian Meteria Medica

**Preparation of Extracts**

The roots were washing with water, dried under shade and finely powdered. The powdered roots (1kg) were extracted with petroleum ether (60-80° C), benzene, ethyl acetate and ethyl alcohol in soxhelt apparatus by simultaneous extraction each for 72 hrs and gave the yield of 2.5 gm, 4.0 gm 3.5 gms, and 2.8 gms respectively. The highest yield of benzene extract was chosen for the isolation of ecdysteroid.

**Preliminary Phytochemical Investigation**

The Determination of active phytochemical constituents was carried out for Petroleum ether, benzene, ethyl acetate, ethyl alcohol extracts using the standard procedures.[5]

**Isolation of Ecdysteroid**

Pure compound is achieved by column chromatography (silica gel 60- 120 mesh, Merck, India, 100gms, 60 cm) eluted with a step – gradient of n-hexane and chloroform. The white solid pure compound obtained by combined mother liquors were concentrated the eluent (100% chloroform) fraction of 224 – 249 (each fraction 50ml) this homogeneous residue was tested by TLC and respond positively for Liebermann - burchard reaction (Characteristic test for steroid).

**Thin Layer Chromatography**

All the extracts were screened for the presence of different phytochemicals employing thin layer chromatographic (TLC) techniques. The Precoated Thin layer plates with silica gel G (Merck, 0.25 mm thickness) were used. TLC of isolated compound (2- deoxy 20 hydroxy 22 cinnamoyl ecdysone 3,25 diacetate) was carried out on silicagel – G using solvent systems $CHCl_3$: methanol (8:2, $R_f$ Value – 0.520) (or) Toluene: $CHCl_3$: Ethanol (4:4:1, $R_f$ value 0.580). The ecdystroid was detected by using anisaldehyde in $H_2SO_4$ spray reagent heated at 100°C for 5- 10 mins and the violet colour was observed.[6]

**Spectral Analysis**

Melting point was determined using Biochem melting point apparatus. UV spectrum was recorded on Perkin Elmer EZ 301 spectrometer. A FT-IR spectrum was recorded on Shimadzhu 8400 Spectrometer. NMR Spectra were recorded on Bruker AV300 ultra shield instrument at $27^0$C using standard Bruker micro programs. The solvent was $CDCl_3$ with tetra methyl silane as an internal standard. A mass spectrum was recorded on JEOL GC mate mass spectrometer.

**Antibacterial Activity**

***Kirby-Bauer Method***

A standardized filter paper disc –agar diffusion procedure known as the Kirby-Bauer method was frequently used for to determine the drug (extracts) susceptibility of microorganism isolated from infectious process. This method allows for fast determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent cause into the medium surrounding the disc.

**Microorganisms**

The gram positive and gram negative (*Bacillus subtilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa*) bacteria were used as 0.85% of saline suspension.

**Growth Medium**

Medium was performed on the plates of Muller hinton agar medium (13.3 gm in 350ml) sterilized for 15 mins at $121^0$ C; approximately 20 ml of this medium was added to each sterile petridish.

**Test Drug**

Petroleum ether, benzene, ethyl acetate, ethyl alcohol and isolated compound were dissolved in DMSO (1000µg/10 µl).

**Testing Procedure**

Agar plates were placed in a incubator heated to $37^0$C. Plates were labelled with the name of the test organism to be inoculated and covered. Using sterile technique, all agar plates were inoculated with their respective test organism as follows

A) A sterile cotton swap was dipped in to a well-mixed saline test culture and a excess inoculum removed by pressing the saturated swap against the inner wall of the culture tube.

B) The entire agar surface streaked horizontally, vertically around the outer edge of the plate to ensure the heavy growth over the entire surface using by swap method

C) All culture plates allowed drying for about 5 minutes

D) Using the senci disc dispenser was applied the antibiotic disc (Chloramphenicol 30 µg) and simultaneously test drug disc were placing with a dispenser separately over the agar surface and simultaneously pressing the plunger depositing the disc onto the agar surface.

E) All plate cultures were incubated in an inverted position for 24-48 hrs at $37^0$C

F) After the incubation period the diameter of the zone of inhibition was measured in millimetre.[7]

## RESULTS AND DISCUSSION

The petroleum ether (60-80° C), benzene, ethyl acetate and ethyl alcohol extract gave the yield of 0.25%, 0.4%, 0.35%, and 0.28% respectively. The preliminary phytochemical investigation has shown the presence of steroid in all extracts. The saponins are present in ethylacetate and ethyl alcoholic extracts. The carbohydrate was present only in alcoholic extract. The phytochemical investigation results are shown in Table 1.

**Table 1:** Results of Preliminary Phytochemical investigation

| Name of the Test | Pet ether Extract | Benzene Extract | Ethyl acetate Extract | Ethyl alcohol Extract |
|---|---|---|---|---|
| Carbohydrates | -- | -- | -- | -- |
| Proteins | -- | -- | -- | -- |
| Steroids | + | + | + | + |
| Saponins | -- | -- | + | + |
| Glycosides | -- | -- | -- | -- |
| Flavonoids | -- | -- | -- | -- |
| Alkaloids | -- | -- | -- | -- |

The white solid pure compound obtained by column chromatography with the eluent of 100% chloroform fraction of 224 – 249 (each fraction 50ml) this homogeneous residue was tested by TLC and respond positively for Liebermann - burchard reaction (Characteristic test for steroid).

Isolated compound ( 2 deoxy 20 hydroxy 22 cinnamoyl ecdysone 3, 25 diacetate) is a white amorphous powder, melting point - 220 ± 1° C, Soluble in chloroform. The isolated ecdysteroids gave a single spot on TLC plate and a positive reaction (Green colour) to Liebermann burchard reaction.

The UV spectrum of a methanol solution of isolated compound showed strong absorption at 231 nm. It shows that the isolated ecdysteroid contain$\alpha$, $\beta$ unsaturated carbonyl compound (7- ene 6- one chromophore).[8]

The UV spectrum of isolated compound is shown **Figure 3.**

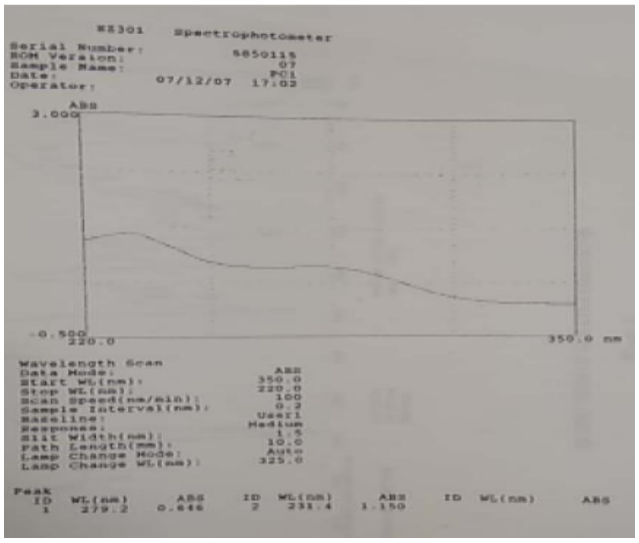


**Figure 3:** UV spectrum of isolated compound

The I.R Spectrum of substance contains absorption bands due to hydroxyl groups ( 3418 $cm^{-1}$) and to an $\alpha$, $\beta$ unsaturated keto grouping ( 1640 $cm^{-1}$)[8]. In addition absorption bands were detected at 1722$cm^{-1}$ and 1269$cm^{-1}$, 1178 $cm^{-1}$( ester) 1601$cm^{-1}$ and, 757 $cm^{-1}$ (aromatic ring) Permitting the assumption that there was an ester group of aromatic nature in ecdysteroid.[9] The IR spectrum of isolated compound and its data are shown Figure 4 and Table 2.

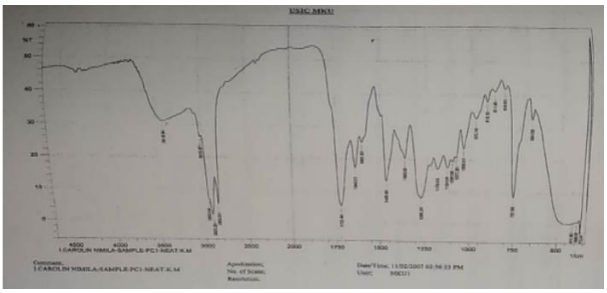


**Figure 4:** IR spectrum of isolated compound

**Table 2:** IR spectrum data of isolated compound

| Wave Number $cm^{-1}$ | Group Assignment |
|---|---|
| 3418 | OH stretching |
| 3013 | Aromatic CH stretching |
| 2955, 2921, 2852 | CH stretching (CH3,CH2) |
| 1722 | C=O stretching (Aryl ester) |
| 1640 | $\Delta^7$-6 keto group |
| 1601 | Aromatic C=C stretching |
| 1465,1360 | CH bending (CH3,CH2) |
| 1269, 1178, 1124, 1095, 1077, 1035 | C-O stretching |
| 972, 913, 871, 818 | CH bending |
| 757 | Aromatic C-C bending |

This was also indicated by the signals of five aromatic protons at $\delta$ 6.69, 7.51, 7.70 ppm (3H) and $\delta$ 8.09 ppm (2H) were assigned for the five protons of benzene ring[9] of cinnamic acid and the signlas at two olefinic protons of cinnamic acid 6.69 and 7.51 ppm were assigned for H-$\alpha$ and H-$\beta$ of olefinic proton of a cinnamic acid[10]. The presence of ring current causes the proton attached to the ring to appear in the range of 7-8 ppm. Which were assigned for five (H) aromatic protons of the benzene ring. The$^1$H NMR spectrum of isolated compound are shown **Figure 5**.

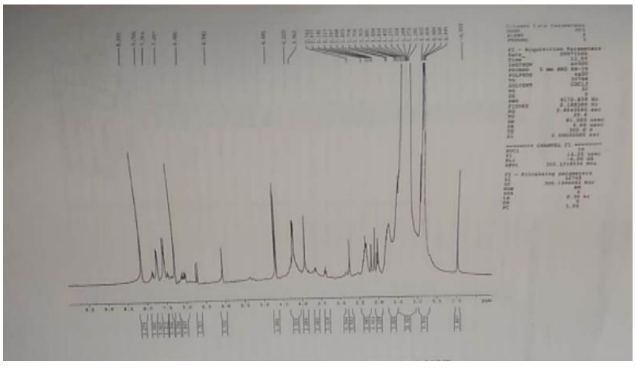


**Figure 5:** $^1$H NMR spectrum of isolated compound

A comparision of $^{1}H$ NMR spectra of isolated compound with 2 deoxy 20 hydroxy ecdysone[11] and 20 hydroxy ecdysone[12] showed that the signal of C-3, C-22, C-25 had undergone a considerable downfield displacement. This fact permitted the assumption that in isolated compound the cinnamic acid esterified that hydroxy group at C-22 and acetyl group at C-3 and C-25. The $^{13}$CNMR spectrum of isolated compound are shown **Figure 6**.

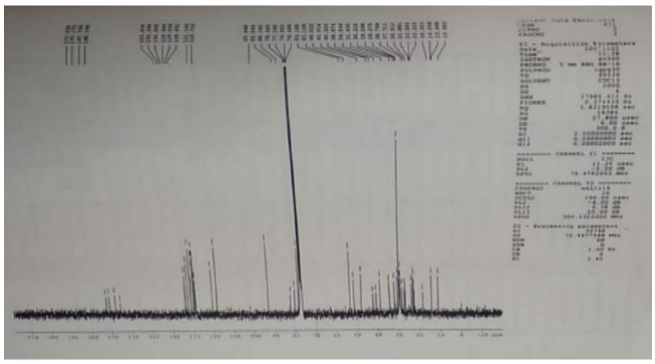


**Figure 6:** $^{13}$C NMR spectrum of isolated compound

The signals $^{13}$CNMR, DEPT 90 and DEPT 135 confirms the presence of seven methyl ($CH_3$), Nine methelene ($CH_2$) Thirteen Methine (CH) and eleven quartinary carbon in the isolated compound. The $^{13}$C NMR and $^{1}$H signals in addition to the 20- hydroxy ecdysone confirm the presence of cinnamic acid and two acetic acid esters in the isolated compound. The DEPT 90 and DEPT 135 spectrum of isolated compound are shown **Figure 7&8**.

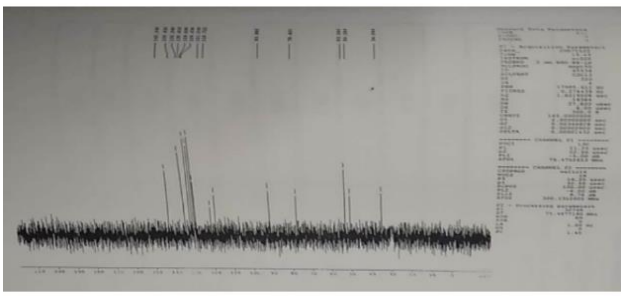


**Figure 7:** DEPT 90 spectrum of isolated compound

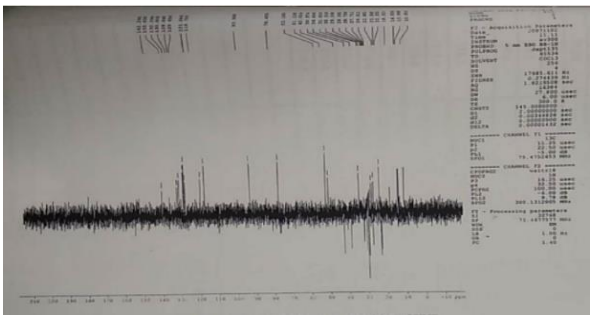


**Figure 8:** DEPT 135 spectrum of isolated compound

The peaks absorbed in the mass spectrum of isolated compound of a molecular ion with m/z 679 ($M^+$) and its fragment ion with m/z 661 ($M^+$ - $H_2O$) 618 ($M^+$- $H_2O$-$C_2H_3O$) 575 ($M^+$ - $H_2O$-$2C_2H_3O$), 444 ($M^+$ - $H_2O$-$2C_2H_3O$-$C_8H_7$), 428, 412, 234, 149, 148, 131 and 77 were observed.

The ions at 618, 575, 444, indicates the presence of two acetyl and cinnamoyl esters in the compound. The peaks of m/z 234 and 347 confirms that the presence of aromatic ring in the side chain[13]. The intense peaks of the ions with m/z 149 ($C_9$ $H_9$ $O_2$) 148 ($C_9H_8O_2$) 131 ($C_9H_7O$) and 77 ($C_6H_5$) which are characteristics fragmentation of cinnamic acid and also confirms the presence of ester of cinnamic acid[14]. The Mass spectrum of isolated compound are shown **Figure 9.**

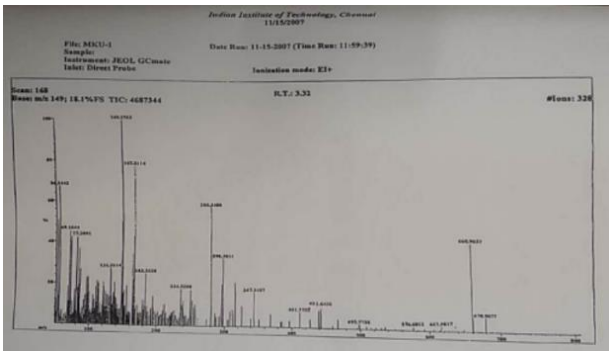
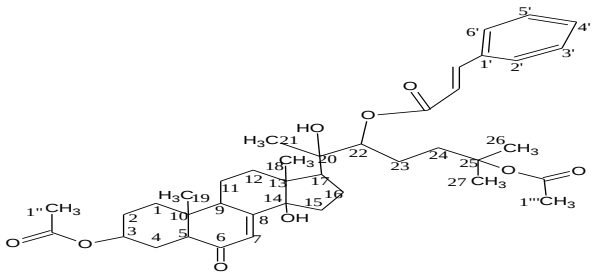


**Figure 9:** Mass spectrum of isolated compound

The down field shift of the H-22 proton confirms the presence of cinnmoyl group at H-22, and also the mass fraction at m/z 234 and 347 also confirms the presence of aromatic ester group present in the side chain. The down field shift H-3 confirms the presence of acetic acid ester at C-3 position, this was also confirmed by the presence of intense peak of mass fraction at 618 ($M^+$ - $H_2O$-$C_2H_3O$), and also presence of mass fragment ion peak at m/z) 575 ($M^+$ - $H_2O$-$2C_2H_3O$) shows the presence of one more acetyl group at C-25. Thus based on $R_f$ value, UV, IR, $^{1}$HNMR, $^{13}$C NMR, DEPT 90, DEPT 135 and EI-MS spectral tudies the isolated compound has been characterized as 2-deoxy, 20-hydroxy, 22-cinnamoyl ecdysone, 3, 25 diacetate and it is shown in **Figure 10.**



2-deoxy,20-hydroxy,22 cinnamoyl,ecdysone 3,25 diacetate

**Figure 10**

NMR Data$^{1}$H NMR(300 $MH_Z$ $CDCl_3$) δ 1.30 ($H_a$ -1), δ 1.68 ($H_e$ -1), δ 1.65 ($H_a$-2), δ 1.73 ($H_e$ -2), δ 4.22 ($H_e$ -3), δ1.40 ($H_a$ – 4), δ1.73 ($H_e$ -4), δ 2.74 (H -5), δ 6.04 (H – 7), δ 3.91 (H – 9), δ 1.61 ($H_a$ -11), δ 1.77 ($H_e$ -11), δ 1.27 (Ha -12), δ 1.65 ($H_e$ -12), δ 2.11 ($H_a$ -15), δ 1.61 ($H_e$ -15), δ1.86 ($H_a$ – 16), δ.1.77 ($H_e$- 16), δ 2.37 (H-17), δ 4.68 (H-22), δ 1.28 ($H_a$-23), δ 1.70 ($H_e$ – 23), δ 1.80 ($H_a$ -24), δ 1.37 ($H_e$-24), δ 0.84 ($CH_3$ -18), δ 0.86 ($CH_3$ -19), δ 1.24 (($CH_3$-21), δ 0.88 ($CH_3$ -26), δ 0.90 ($CH_3$ -27), δ 2.09 (1" – $CH_3$), δ 2.19 ($CH_3$ – 1"'), δ 7.70 (H -2', 4', 6'), δ 8.09 (H-3', 5'), δ 6.69 (H-α), δ 7.51 (H-β).

The **$^{13}$CNMR (75 MHzCDCL$_3$)**, δ 38.5 ($CH_2$ -1), δ 29.2($CH_2$ -2), δ 78.4 (CH -3), δ 31.8 ($CH_2$ -4), δ 53.1 (CH -5), δ121.0 (CH -7), δ 165.1 (C-8), δ 34.0 (CH -9), δ 40.3 (C-10), δ 22.2 ($CH_2$ -11), δ29.5 ($CH_2$ -12), δ 48 (C-13), δ 82.5 (C-14), δ 30.2 ($CH_2$ –15), δ22.5 ($CH_2$-16), δ 51.1 (CH-17), δ14.0 ($CH_3$ -18), δ 18.3 ( $CH_3$ -19), δ 77.3 (C-20), δ 22.8 ($CH_3$ -2'), δ 93.9 (CH -22), δ 26.5 ($CH_2$ -23) , δ 42.0 ( $CH_2$-24), δ 80.2 (C-25), δ 10.8 ($CH_3$ -26), δ13.9 ($CH_3$-27), δ 128.6 (C-1′), δ 129.9 (CH -2′), δ132.2 (CH -3′), δ 130.8 (CH -4′), δ 133.6 (CH -5′), δ 129.6 (CH -6′), δ 118.7 (CH -α), δ 142.2 (β-CH), δ 27.71 ($CH_3$-1''), δ 28.7 ($CH_3$-1‴), δ 167.7 (Acetyl C=0), δ170.1 (acetyl C=0), δ 172.0 (Cinnamic Acid C =0).

**DEPT 90 (75 MHz, CDCL$_3$ )** δ 34.04 (CH -9), δ 51.12 (CH -17), δ 53.1 (CH-5), δ 78.4 (CH-3), δ 93.9 (CH -22), δ118.7 ( CH-α), δ 121.0 ( CH-7), δ 129.6 (CH -6′), δ129.9 (CH -2′), δ130.8 (CH-4′), δ 132.2 (CH-3′), δ 133.6 (CH -5′), δ 142.2 (CH -β)

**DEPT 135 (75 MHz , CDCL$_3$)** δ34.04 (CH -9), δ 51.12 (CH-17), δ 53.1 (CH-5), δ 78.4(CH-3) , δ93.9 (CH-22), δ118.7 ( CH-α) , δ121.0 (CH-7), δ129.6 (CH-6′), δ 129.9 (CH-2'), δ 130.8 ( CH -4′), δ132.2 (CH-3'), δ133.6 (CH-5'), δ142.2 (CH-β), δ 22.2 ($CH_2$ -11), δ 22.5 ($CH_2$ -16) δ26.5 ($CH_2$ -23), δ 29.2 ($CH_2$-2), δ 29.5 ($CH_2$-12), δ 30.2 ($CH_2$-15), δ 31.8($CH_2$ -4), 38.5 ($CH_2$-1), δ 42.0 ( $CH_2$-24), δ10.8 ($CH_3$-26), δ13.9 ($CH_2$-27), δ14.0 ($CH_3$-18), δ18.3 ($CH_3$-19), δ 22.8 ($CH_3$-21), δ 27.7 ($CH_3$-1''), δ 28.7 ($CH_3$-1‴).

### Antibacterial Study

In Disc diffusion method, isolated compounds and the extracts such as petroleum ether, benzene, ethyl acetate and ethyl alcohol were does not produced significant zone of inhibition up to the concentration of (1000µg/10 µl) against the microorganism such as *Bacillus subtilis, Proteus vulgaris, Escherichia coli* and *Pseudomonas aeruginosa.*

## CONCLUSION

Several studies reported antioxidant, free radical-scavenging and neuroprotective effects of 20-hydroxyecdysone. We are currently focusing our research, to test the *Polycarpaea corymbosa* root extracts and the isolated compound (2-deoxy, 20-hydroxy, 22-cinnamoyl ecdysone, 3, 25 diacetate) for *in-vitro* inhibitory activity on toxic venom enzymes like phosphomonoesterase, phosphodiesterase, acetylcholinesterase, hyaluronidase etc would provide a potential treatment against snake bite.

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