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Isolation and antibacterial activity of terpenoid from *Bougainvillea glabra* choicy leaves

J. Mariajancyrani[1*], G. Chandramohan[1], Saravanan[2] and A. Elayaraja[1]

[1]*Department of Chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India*
[2]*Department of chemistry, Kings College of Engineering, Punalkulam, Thanjavur, Tamil Nadu, India*

ABSTRACT

This study reports the isolation and antibacterial activity of terpenoid extracted from the leaves of Bougainvillea glabra choicy. The isolation of organic compounds was done using simple chromatographic technique. Compound characterization using various spectroscopic techniques identified the final isolated compound as Oleananoic acid acetate. This is the first report of the presence of terpenoid in the leaves of Bougainvillea glabra choicy. Antibacterial activity of these compounds was against Gram positive, Gram negative bacterial strains by observing the zone of inhibition. Antibacterial activity was done by well diffusion method at a concentration of 25µg/25µl, using ethanol as the control. As, a result terpenoid showed excellent antibacterial activity. The method of isolation is simple, cost effective and efficient.

Key words: Antibacterial activity, *Bougainvillea glabra choicy*, Chromatographic technique, Oleananoic acid acetate, spectroscopic technique.

INTRODUCTION

Bougainvillea glabra is a good choice for plant around the house for security to keep people from climb thorns. It makes lovely colors to spread on walls and fences. The common name of Bougainvillea is Glory of garden, it is originated from South America and it also popular plant in Southern California, Florida [1]. Bougainvillea flowers are ranging from yellow, pink, red, orange, purple and especially white. The varieties of Bougainvillea's include Bougainvillea spectabilis and Bougainvillea harrisi and these plants are mainly grown in decorative purposes in tropical regions [2, 3]. *Bougainvillea glabra* choicy have been used by the variety of disorders like diarrohea, reduce stomach acidity, cough and sore throat [4].

Plant terpenoids can be used enormous for their aromatic qualities. Terpenoids can play an important role in traditional medicines and are under investigation for antineoplastic, antibacterial and other pharmaceutical functions [5]. *Bougain villea glabra choicy* contains large amount of terpenoids, polyphenolic compounds, tannins, cardiac glycosides and anthroquinones[6]. The Bougain villea glabra choicy leaves are used in antimicrobial, anti-diarrhoeal activity, anti hyperglycemic activity, anti-inflammatory activities [7]. The aim of this study to isolate and evaluate antibacterial activity of Oleananoic acid acetate from ethyl acetate fraction.

MATERIALS AND METHODS

Collection, Identification and preparation of plant materials

The leaves of the plant *Bougainvillea glabra choicy* collected from Thanjavur district and authenticated by Dr. John Britto, Rapinet Herbarium, St .Joseph's College, Tiruchirappalli. The leaves were cleaned, dried in shadow and crushed into powder.

Extraction

The powdered sample was extracted with 95% ethanol by using cold method extraction in room temperature for one week. The 95% ethanol extract was filtered, distilled and concentrated to obtain the solid greenish residue. The 95% ethanol extract was further partitioned successively with petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, n-butanol and methanol. The solvents were recovered under reduced pressure.

Isolation

Ethyl acetate soluble part (5.8g) was subjected to silica gel (70-130 mesh) Column chromatography (60cmx4.5cm). The ethyl acetate soluble part eluted gradient with Chloroform, Ethyl acetate, Methanol. The methanol fraction were rechromatographed with Ethyl acetate, Ethyl acetate: Methanol mixtures (4.05:0.05, 4:1), Methanol. The eluents were collected and the progress of separation was noted by micro thin layer chromatography using Ethyl acetate: Methanol (4.75:0.25) solvent system and iodine vapor as detecting agent.

4:1 Ethyl acetate: Methanol fraction were purified and recrystallized by methanol. A White solid powder obtained, which was characterized by spectroscopic studies (IR, NMR, EI-MS, ESI-MS (Negative mode).

Antibacterial activity

Antibacterial activity of Oleananoic acid acetate was done using published protocols [8]. The concentration of the compound was 25µg/mL.

Figure 1: Oleananoic acid acetate

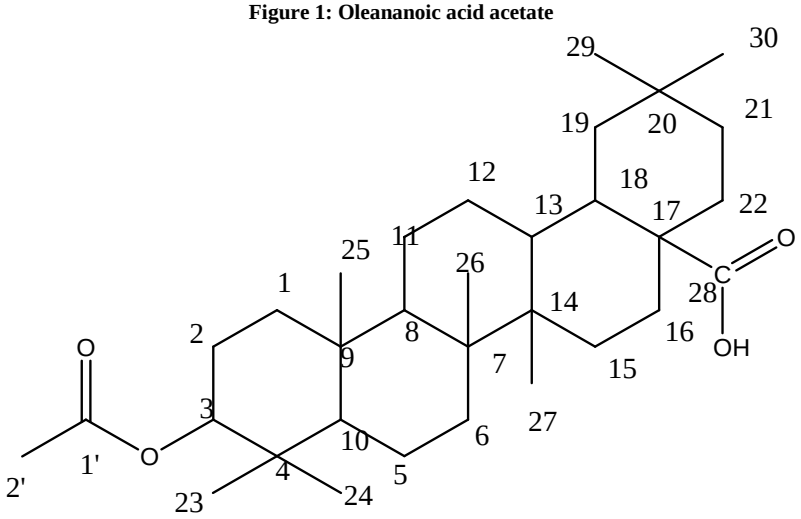

Figure 2: Micro Thin Layer Chromatography (Ethyl acetate: methanol 4.75:0.25 solvent system)

RESULTS AND DISCUSSION

Spectral analysis of isolated compound

The structure of Oleananoic acid acetate as shown in Fig-1. Single spot of Oleananoic acid acetate on Thin layer chromatography as shown in Fig-2. The results of the spectral analysis data were tabulated at Table-1.

Table 1: Spectral Data

Type of Experiment	Unit	Data
IR Perkin Elmer FTIR (450-4000 cm^{-1})	Δ γ□cm $^{-1}$	3313(OH bond), 1589(C=O of acid), 1684 (C=O of ester), 2923 (C-H stretch), 1499, 1299 (CH_3, CH_2 gp), 1021(Cyclo alkane)
'HNMR Bruker (400MHZ)	δppm	4.161 (H-3), 0.809 (H-5), 1.255 (H-9), 1.85 (H-11), 1.74 (H-18), 0.830 (H-24), 0.688 (H-26), 0,994 (H-27), 0.905 (H-30), 2.134 (O-CH_3)
^{13}CNMR (100MHZ)	δ ppm	28.35 (C-1), 22.38(C-2), 78.52(C-3), 31.33(C-4), 49.56(C-5), 18, 69(C-6), 29.79(C-7), 39.59(C-8), 45.16(C-9), 29.19(C-10), 19.48(C-11), 23.77(C-12), 36.16(C-13), 40.01(C-14), 28.5(C-15), 23.20(C-16), 41.76(C-17), 30.47(C-18), 40.22(C-19), 29.02(C-20), 35.49(C-21), 29.02(C-22), 18.95(C-23), 18.69(C-24), 13.79(C-25) 11.61(C-26), 11.46(C-27), 180.3(C-28), 38.12(C-29), 38.31(C-30), 167(C-1^1), 10.60(C-2^1)
EI-MS(Jeol)	m/z	459(5), 485(5)
ESI-MS (Thermo LCQ Deca XP max. Range m/z=1-2000.	m/z	501(10), 457(7), 485(58)

Table 2: Anti-Bacterial Activity

S.NO.	Name of Pathogens	Control	Zone of inhibition
1	*Streptococcus mitis*	0	19mm
2	*Lactobacillus SP*	0	13mm

Oleananoic acid acetate was obtained white solid which gave positive Lieberman-Burchard test for triterpenoids[9].

IR Spectra

IR spectra showed absorption frequency at 3384, this indicates the presence of (O-H) stretch for hydroxyl group, which was bonded with (C=O) of an acid obtained the signal at 1589. This two support the carboxylic acid (-COOH), functional group at position of C-28. The frequency at 2923 is due to (C-H) stretch for an alkane and absorption showed at 1499, 1299 is due to presence of (-CH_3, CH_2) group in the molecule. The absorption frequency at 1021 signifies cycloalkane.

NMR Spectra

IN 'H NMR spectra, the chemical shift obtained at 4.161 is indicated the (H-3) bonded with oxygen group. The signal at 0.809, 1.255 and 1.74 is due to presence of 'CH' group and signal at 1.85 due to –CH_2 group. The 'HNMR showed shift at 0.830, 0.688, 0.994, 0.905 attribute the –CH_3 groups. The presence of Olean skeleton was confirmed in the ^{13}C-NMR spectrum with the signals in the region δ 11.46-38.31ppm at 26 and at 23.77 attributed to seven methyl groups and absence of double bond at the position of C-12, C-13. ^{13}C NMR shows shift at 180.3 corresponds to (-COOH) bond at the position of C-28 and 167, 10.60 corresponds to (C=O) linkage at position C-1^1, C-2^1.

Mass spectroscopy

Interpretation of molecular formula predicted mainly on the basis of mass spectra (EI-MS, ESI-MS). The mass spectra of the compound were matched with mass spectra obtained from metlin software [10]. In EI-MS, the molecular ion not observed but the molecular ion (M^++H) of compound was observed at m/z-501(10) in the ESI-MS respectively showing its molecular formula $C_{32}H_{52}O_4$ and fragmented peaks at for EI-MS- 459 (5), 485 (5) and for ESI-MS- 457(7), 485(58). IR absorption band at is due to C-H stretch for an alkane. This account for the high degree of saturation of the molecule. This also supported by ^{13}C NMR, the signal obtained at 36.6& 23.77. Based on the above characterization and by comparing with other similar compounds, the isolated compound is Oleananoic acid acetate. It was good agreement with literature data [11-14].

Antibacterial activity

The Result of the zone of inhibition of oleananoic acid acetate against selected micro-organism was given in Table-2. The diameter of inhibition zones was measured in mm and the result were recorded inhibition zones with diameter less than 12mm were considered ad having no antibacterial activity. Diameters between 12 and 16mm were considered moderately active and these with ≥ 16mm were considered highly active. To find new antibacterial compound is a continuous effort of screening of antibacterial activity of plant extracts [15]. The antibacterial activity of *Bougainvillea leaves* were reported by Rani et al [8]. It was evident that the present study results were confirmed the antibacterial inhibition against two organisms.

CONCLUSION

Compound characterization using various spectroscopic techniques identified the final isolated compound as oleananoic acid acetate and it showed excellent anti-bacterial activity. The method of isolation is simple, cost effective and efficient. This is the first report of the presence of terpenoid in the leaves of *Bougainvillea glabra choicy*.

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