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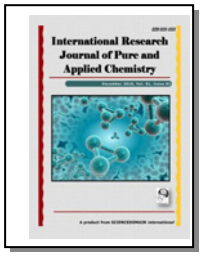

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Study on Isolation and Comparison of the Chemical Compositions of *Cissus adnata* Roxb. leaves and *Smilax lanceaefolia* Roxb. Roots and Their Free Radical Scavenging Activities

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ABSTRACT

The extracts from *Cissus adnata* Roxb. leaves and *Smilax lanceaefolia* Roxb. roots have been used as medicinal remedies to promote dissolution and expulsion of urinary calculi. From methanol extract of the leaves of *Cissus adnata*, three compounds, namely, **CSM1** (a triterpenoid), **CSM2** (a flavonoid) and **CSM3** (apigenin) were isolated. Whereas six compounds, namely, β-sitosterol, β-siosterol-D-glucoside, **SL1** (a flavonoid), **SL2** (a terpenoid), **SL3** (a saponin) and **SL4** (a glycoside) were isolated from the roots of *Smilax lanceaefolia*. A comparative study of the chemical components present and antioxidant activities of *Smilax lanceaefolia* roots and that of *Cissus adnata* leaves is also reported by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β-Carotene-linoleate methods. The comparative study of the chemical components present and antioxidant activities of the two plants showed that the isolated compounds were found to have moderate to high free radical scavenging activities.

Keywords: Isolation, Comparison, Cissus adnata, Smilax lanceaefolia, Antioxidant;

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1. INTRODUCTION

In the recent past, there has been a global trend towards revival of interest in the medicinal plants. It is because the herbal medicines are believed to be comparatively safer than that most of the modern drugs. Moreover, they are economical and environmentally better suited for local conditions. In a developing country like India, the utilization of natural products and their improved sustainability is a distinct advantage over the developed countries. The natural products would remain very important for affordable health care as our national imperative in view of the implementation of the Dunkel Draft (the GATT agreement and the WTO) and patent protection. While medicinal plants and traditional medicine are integral parts of the health delivery system in developing societies like India, the developed countries too have in recent times shown interest to the use of traditional medicinal systems that involve the use of herbal drugs and remedies. In addition to purified plant derived drugs, there is an enormous market for crude herbal medicines as dietary supplements, and for therapeutic purposes in both the developed and developing countries of the world. The development of clinically effective anti-cancer agents, such as taxol and the discovery of potential anti-AIDs agents, such as michellamine B, demonstrate the significance of plants as sources of potential new drugs. Almost all medicinal herbs act on the kidneys. Diuretic plants cause an increase in the urine volume promoting the function of elimination of waste substances which kidneys performed. The diuretic properties of plants are also enhanced by the water with which herbal teas or herbal soups are prepared for oral administration. Uric acid and urea are two of the most toxic substances our body continuously produces and they are eliminated through urine. Urine contains many mineral salts. Some plants are highly effective in increasing the solubility of mineral salts present in the urine. When they are not dissolved, these salts form precipitations in the kidneys called kidney stones or calculi. There are plants which can even dissolve kidney stones. *Cissus adnata* Roxb. and *Smilax lanceaefolia* Roxb. are believed to promote dissolution and expulsion of urinary calculi.

The leaves of *Cissus adnata* (*Vitaceae* family) are prescribed in urinary troubles due to calculi; crushed leaves of the plant are also applied to fractured bones by the local people of North-east India. Tuber decoction is alterative, diuretic and blood purifier. Powdered roots are antiseptic, applied to wounds, cuts and fractures. Compounds **CSM1** (a triterpenoid), **CSM2** (a flavonoid) and **CSM3** (apigenin) were isolated from the methanol extract of *Cissus adnata* leaves. The roots of *Smilax lanceaefolia* (*Liliaceae* family) are used medicinally by the people of North-east India including as tea substitute. It is believed that the roots exert a preventive action which is notable on renal calculi formation, and the roots even can dissolve, in some cases, already formed calculi. The root is believed to act as a blood purifier and regulate the menstrual cycle of women. The root juice is used in rheumatic pains and the refuse after extraction of juice is used as poultice over the affected parts. Root decoction is also prescribed in stomach pains. From the methanol extract of *Smilax lanceaefolia* roots, six compounds were isolated, namely, β-sitosterol, β-siosterol-D-glucoside, **SL1** (a flavonoid), **SL2** (a terpenoid), **SL3** (a saponin) and **SL4** (a steroidal glycoside).

Herein, we report the isolation of active principles from *Cissus adnata* leaves and *Smilax lanceaefolia* roots. A comparative study of the chemical components present and antioxidant activities of the two plants which are used in treatment of problems arising due to renal calculi formed inside kidney and urinary tract by the locale people of north-east India are also reported.

2. MATERIALS AND METHODS

2.1 GENERAL

Melting points were determined by capillary tubes and are uncorrected. Infrared (IR) spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer. All samples were run as a thin film (produced by evaporation of a chloroform solution) on a sodium chloride plate. Absorption maxima were recorded in wave numbers (cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on Varian unity 500 (500 MHz), Bruker AC-300 and Varian XL (300 MHZ) spectrometers. ^{13}Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker AC-300 and Varian XL (75 MHz) spectrometers. Residual non-deuterated solvent was used as an internal reference and all chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm) downfield from tetramethyl silane (TMS). All samples were run in deutero-chloroform ($CDCl_3$) as solvent unless otherwise stated. Mass spectra were recorded on a Kratas concept-IS mass spectrometer couples to a Mach 3 data system, or on a Jeol-D 300 mass spectrometer.

2.2 PLANT MATERIALS

Collection of the plant parts for the two plants was done during 2009-2010. The herbarium of the plants were identified and kept in the museum of the University. The samples were washed, dried, chopped and powdered. Fresh parts of the plants which had been cleaned were used only to determine the moisture content.

2.3 DETERMINATION OF CHEMICAL COMPOSITION

2.3.1 Analysis of Feeds: Wet Digestion Method (Hart and Fisher, 1971)

This method describes the determination of copper, zinc, sodium, potassium, magnesium, cadmium, lead, calcium and manganese in feeds. The feed samples are usually digested in glass beakers using a mixture of nitric acid and perchloric acids, filtered into a volumetric flask and then brought to volume with deionized water. Instead of using beakers, samples can be digested directly in volumetric flasks to eliminate losses; from spattering and sample transfer.

2.3.2 Typical Analytical Procedure

One gram of the sample was weighed into a 500 mL beaker and to it, 20 mL of conc. HNO_3 was added, and covered with a watch glass. It was boiled gently for 2 h to oxidize all the easily oxidizable material. The solution was then cooled and 10 mL of 70% $HCLO_4$ was added slowly. It was boiled again gently until the solution was nearly colourless, taking care that the solution should not be allowed to go to dryness. It was cooled, deionized water was added, filtered and diluted to 100 mL with deionized water.

It is to be noted that conc. HNO_3 should always be added to the feed samples and allow this mixture to digest before adding $HCLO_4$. $HCLO_4$ can react explosively with untreated organic matter.

2.3.3 Calculation

Concentration of the element in mg per 100g of the sample was calculated by the following relationship,

$$\text{Conc. of the element (mg/100g of the sample)} = \frac{C \times V}{10\ W}$$

where C is the spectrophotometric reading given by the spectrophotometer, V is the final volume of dilution (100 mL) and W is the original sample weight taken.

2.4 DETERMINATION OF ANTIOXIDANT ACTIVITY

The total antioxidant activities were determined by DPPH free radical scavenging activity and β-Carotene-linoleate methods.

2.4.1 Analysis of antioxidant activity by scavenging of 1,1- diphenyl-2-picrylhydrazyl (DPPH) Method (Blossi, 1958)

About 2.8 ml of methanolic solution DPPH (45μg/ml sigma) were rapidly mixed with 200μl of a methanolic sample in a cuvette placed in the spectrophotometer. The absorbance at 515nm was measured after 5 mins. BHT (Butylated hydroxy toluene) solution (125μg/ml sigma) was used as a reference corresponding to 100% radical scavenging activity.

Radical scavenging activity percentage was calculated as

Radical scavenging activity (%) = $\{(A_{0(sample)} - A_{test})/(A_{0(ref)} - A_{ref})\}$ x 100,

where A_o are the initial absorbance values; and A_{ref} and A_{test} are the absorbance values after 5 mins with BHT and sample solution respectively.

2.4.2 Analysis of antioxidant activity by β-Carotene-linoleate model system (Jayaprakasha et al., 2001)

A solution of β-Carotene was prepared by dissolving 2 mg of β-Carotene in 10 ml of chloroform. 2ml. of this solution were pipetted into a 100ml. R.B. flask. After chloroform was removed under vacuum, 40mg. of purified linoleic acid, 40 mg of TWEEN 80 emulsifier and 100ml of aerated distilled water were added to the flask with vigorous shaking. Aliquots (2.8ml) of this emulsion were transferred into different test tubes containing different extracts. BHT is used as reference. Absorbance is recorded at 470 nm using spectrophotometer.

2.5 EXTRACTION AND ISOLATION

Dried and powdered *Cissus adnata* leaves (1.25 kg.) and *Smilax lanceaefolia* roots (1.0 kg.) were extracted with petroleum ether, chloroform and methanol, successively. The petroleum ether and chloroform extracts for both the plants were found to contain fats and oils, and were not sufficient for further analysis. Three compounds were isolated from the methanol extract of *Cissus adnata* leaves [Kongbrailatpam, B.D. (2010)] and six compounds were isolated from the methanol extract of *Smilax lanceaefolia* roots (Laitonjam and Kongbrailatpam, 2010).

2.5.1 Onocer-7-ene-3α,21β-diol, CSM1

Colourless crystals, m.p. 200-202°C; IR (KBr)**:** ν_{max} 3352, 2957, 1460, 1072, 1026 cm^{-1}**;** ^{1}H NMR ($CDCl_3$) **:** δ_H 0.71 (3H, s), 0.81 (3H, s), 0.86 (3H, s), 0.94 (3H, d, *J* = 6.9 Hz), 1.03 (3H, s), 1.27 (3H, s), 1.52 (3H, s), 2.02 (3H, s), 3.72 (1H, d, *J* = 4.3 Hz), 3.86 (1H, d, *J* = 4.3 Hz), 4.41 (1H, d, *J* = 9.4 Hz, CH=), 4.73 (1H, s, OH), 5.38 (1H, s, OH).

2.5.2 Adnatoside (Apigenin 8-C-α-D-glucopyranoside), CSM2

White amorphous powder; m.p. 258°-259°C; IR (KBr): ν_{max} 3379, 3242, 1655, 1570, 1364, 1099, 829 cm^{-1}; ^1H NMR (DMSO-d_6) : δ_H 2.51 (2H, s, CH_2), 3.76-3.86 (3H, m, glucosyl H), 4.57-4.92 (3H, m, glucosyl H), 5.03 (1H, br s, H-1"), 6.17 (1H, s, H-6), 6.79 (1H, s, H-3), 6.92 (1H, d, *J* = 9.0 Hz, H-3′/H-5′), 8.03 (1H, d, *J* = 9.0 Hz, H-2′/H-6′), 10.42 (1H, s, 7-OH), 13.16 (1H, s, 5-OH); ^{13}C NMR (DMSO-d_6): δ_C 162.52 (C – 2), 102.38 (C – 3), 182.04 (C – 4), 161.07 (C – 5), 98.07 (C – 6), 163.9 (C – 7), 104.53 (C – 8), 155.94 (C – 9), 103.96 (C – 10), 121.54 (C – 1'), 128.92 (C – 2'), 115.77 (C – 3'), 160.34 (C – 4'), 115.77 (C – 5'), 128.92 (C – 6'), 73.30 (C – 1"), 70.77 (C – 2"), 78.57 (C – 3"), 70.46 (C – 4"), 81.76 (C – 5"), 61.23 (C – 6"); Mass ($C_{21}H_{20}O_{10}$): m/z (%) 433 ([M+1], 25%), 415 ([M-17], 40), 397 ([M-35], 50), 314 (A_1, 20), 313 (A_1-H, 100), 283 (30), 270 ([M-Glucose], 10), 242 ([M-Glucose-CO], 5).

2.5.3 SL1

White amorphous powder ; m.p. 180 – 182°C; IR (KBr); ν_{max} 3389, 1643, 1379, 1296, 1070, 1032, 820, 772 cm^{-1}; ^1H NMR (CD_3OD) : δ_H 1.20 (3H, d, *J* = 6.3 Hz, Me – 6"), 3.33 (1H, t, *J* = 9.6 Hz, H – 4"), 3.55 (1H, m, H – 5"), 3.68 (1H, d, *J* = 11.4 Hz, H – 3), 4.05 (1H, dd, *J* = 9.6, 3.3 Hz, H – 3"), 4.27 (1H, dd, *J* = 3.3, 1.5 Hz, H – 2"), 4.60 (1H, d, *J* = 11.4 Hz, H – 2), 5.11 (1H, d, *J* = 1.5 Hz, H – 1"), 5.91 (1H, d, *J* = 2.0 Hz, H – 8), 5.92 (1H, d, *J* = 2.0 Hz, H – 6), 6.81 (1H, s, H – 6'), 6.87 (1H, s, H – 5'), 6.98 (1H, s, H – 2'); ^{13}C NMR (CD_3OD) : δ_C 83.9 (C – 2), 78.5 (C – 3), 195.9 (C – 4), 165.4 (C – 5), 96.3 (C – 6), 168.5 (C – 7), 97.4 (C – 8), 164.0 (C – 9), 102.1 (C – 10), 129.1 (C – 1′), 115.5 (C – 2′), 147.3 (C – 3′), 116.3 (C – 4′), 146.4 (C – 5′), 120.5 (C – 6′), 102.4 (C – 1″), 71.7 (C – 2″), 72.1 (C – 3″), 73.8 (C – 4″), 70.5 (C – 5″), 17.8 (C – 6″); Mass (FAB+) *m/z* (%) : 451 [M + H], 473 [M + Na], 511 [M + Na + K], 901 [2M + H], 923 [2M + Na], 948 [2M + 2Na + 2H], 985 [2M + 2Na + K].

2.5.4 Aglycone (Neotigogenin), SL2

White amorphous powder; m.p. 134°–136°C; IR (KBr); ν_{max} 3398, 2948, 2901,1648, 1456,1377,1055, 918,459 cm^{-1}; ^1H NMR ($CDCl_3$: CD_3OD = 4:1): δ_H 0.79 (3H, s, H – 18), 0.92 (3H, d, *J* = 7.5 Hz, H –27), 0.96 (3H, d, *J* = 8.0 Hz, H – 21) , 1.02 (3H, s, H –19), 1.07 (2H, m, H – 24), 1.14 (2H, m, H – 4), 1.13 (1H, m, H – 24), 1.17 (2H, m, H – 15), 1.18 (1H, m, H – 14), 1.30 (2H, m, H – 2), 1.46 (1H, m, H – 5), 1.49 (2H, m, H – 7), 1.52 (2H, m, H – 6), 1.54 (1H, m, H – 8), 1.58 (1H, m, H – 9), 1.63 (2H, m, H – 12), 1.64 (2H, m, H – 11), 1.67 (2H, m, H – 26), 1.86 (1H, m, H – 12), 2.01 (1H, m, H – 20), 2.28 (2H, m, H – 1), 3.48 (2H, m, H – 3), 4.40 (2H, m, H –23), 4.42 (2H, m, H – 16), 5.35 (1H, brs, 3 – OH); ^{13}C NMR ($CDCl_3$) : δ_C 37.189 (C – 1), 31.578 (C – 2), 71.694 (C – 3), 42.232 (C – 4), 140.760 (C – 5), 121.394 (C – 6), 32.014 (C – 7), 31.397 (C – 8), 50.015(C – 9), 36.605 (C – 10), 20.842 (C – 11), 39.748 (C – 12), 40.225 (C – 13), 56.482(C – 14), 31.808(C – 15), 80.793 (C – 16), 62.043(C – 17), 16.259 (C – 18), 19.394 (C – 19), 41.566(C – 20), 14.499(C – 21), 100.275 (C – 22), 31.348(C – 23), 28.756 (C – 24), 30.262(C – 25), 66.815 (C – 26), 17.107 (C – 27).

2.5.5 Neotigogenin 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyrano-side, SL3

Amorphous powder; IR (KBr); ν_{max} 3450, 2950, 1470, 1455, 1385, 1340, 1275, 1265, 990, 975, 920, 900, 850, 810 cm^{-1}; ^1H NMR (CD_3OD) : δ_H 0.67 (3H, s, H – 18), 0.81 (3H, s, H –19), 1.08 (3H, d, *J* = 7.1 Hz, H –27), 1.15 (3H, d, *J* = 6.9 Hz, H – 21) , 1.64 (3H, d, *J* = 6.2 Hz, H – 6") , 5.00 (1H, d, *J* = 7.6 Hz, H – 1') , 5.53 (1H, br s, H – 1"); ^{13}C NMR ($CDCl_3$) : δ_C 37.2 (C – 1), 30.1 (C – 2), 77.7 (C – 3), 35.0 (C – 4), 44.6 (C – 5), 29.0 (C – 6), 32.4 (C – 7), 35.3 (C – 8), 54.4(C – 9), 35.9 (C – 10), 21.3 (C – 11), 40.2 (C – 12), 40.8 (C – 13), 56.4 (C – 14), 32.1(C – 15), 81.2 (C –

16), 62.9(C – 17), 16.6 (C – 18), 12.3 (C – 19), 42.5(C – 20), 14. 9(C – 21), 109.7 (C – 22), 26.2(C – 23), 26.4 (C – 24), 27.6 (C – 25), 65.1 (C – 26), 16.3 (C – 27), 102.4 (C – 1′), 75.3 (C – 2′), 78.7 (C – 3′), 72.1 (C – 4′), 77.1 (C – 5′), 68.6 (C – 6′), 102.7 (C – 1″), 72.4 (C – 2″), 72.8 (C – 3″), 74.1 (C – 4″), 69.8 (C – 5″), 18.7 (C – 6″); Mass (FAB+) *m/z* (%) : 747 [M+Na], 725 [M+H].

2.5.6 Stigmasterol glucoside, SL4

Pale white amorphous powder; m.p. 265°-267°C (decomp.); IR (KBr): ν_{max} 3408, 2959, 2933, 2360, 1582, 1165, 1073, 800 cm^{-1}; ^1H NMR ($CDCl_3$: CD_3OD = 4:1) : δ_H 0.68 (s, H – 18), 0.82 (d, J = 7Hz, H – 27 & 29), 0.85 (d, J = 6Hz, H – 26), 0.87 (s, H – 9), 0.91 (br. , H – 24), 0.93 (d, J = 6Hz, H – 21), 1.01 (s, H – 19), 1.03 (s, H – 15), 1.06 (m, H – 14 & 28), 1.13 (m, H – 17), 1.15 (m, H – 12), 1.48 (m, H – 7), 1.54 (brs, H – 8), 1.56 (m, H – 25), 1.87 (brs, H – 16), 2.37 (m, H – 1), 3.23 (m, H – 2'), 3.35 (m, H – 4'), 3.43 (m, H – 3), 3.58 (m, H – 3'), 3.76 (m, H – 5'), 3.80 (m, H – 6'), 4.39 (d, J = 8 Hz, H – 1'), 5.05 (m, H – 23), 5.14 (m, H – 22), 5.37 (br s, H – 6); ^{13}C NMR ($CDCl_3$: CH_3OD = 4:1) : δ_C 37.6 (C – 1), 32.9 (C – 2), 78.2 (C – 3), 36.2 (C – 4), 139.3 (C – 5), 121.2 (C – 6), 30.9 (C – 7), 30.9 (C – 8), 49.2 (C – 9), 35.7 (C – 10), 220 (C – 11), 38.7 (C – 12), 41.3 (C – 13), 55.7 (C - 14), 25.0 (C – 15), 28.5 (C – 16), 55.0 (C – 17), 10.8 (C – 18), 18.2 (C – 19), 35.1 (C – 20), 18.7 (C – 21), 137.3 (C – 22), 128.9 (C – 23), 44.8 (C – 24), 28.1 (C -25), 17.7 (C – 26), 18.0 (C -27), 23.8 (C – 28), 10.9 (C – 29), 100.1 (C – 1'), 72.5 (C – 2'), 75.4 (C – 3'), 72.5 (C – 4'), 74.8 (C – 5'), 60.6 (C – 6'); Mass : m/z 397 [(M+1) - Glucose] $^+$.

3. RESULTS AND DISCUSSION

3.1 ISOLATION AND CHARACTERIZATION

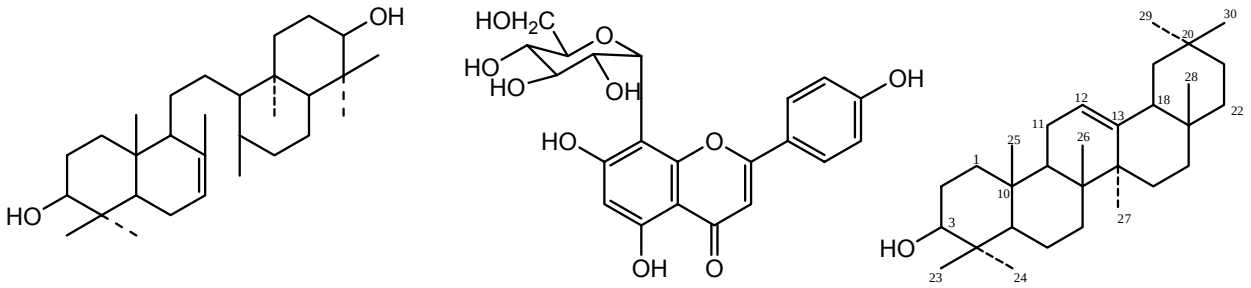
Three compounds were isolated from the methanol extract of *Cissus adnata* Roxb. (Figure 1). When the methanol extract was eluted with chloroform-methanol (96:4), a fraction was separated which was further purified to give compound **CSM1**. Its IR spectrum showed absorptions at 3352, 2957, 1460, 1072, 1026 cm^{-1}. The ^1H NMR spectrum of **CSM1** in $CDCl_3$+MeOD-d_4 showed the presence of six tertiary methyl groups on saturated carbons [δ 0.71, 0.81, 0.86, 1.03, 1.27 and 1.52], one secondary methyl protons on a saturated carbon at δ 0.94 and one olefinic methyl at δ 2.02. The ^1H NMR spectrum also exhibited two deshielded methine protons each bearing a hydroxyl group at δ 3.72 and 3.86; and the hydroxyl protons are observed at δ 4.73 and 5.38. Long-range coupling of the olefinic proton at δ 4.41(d) showed that possibly an olefinic methyl was coupled with the olefinic proton. The presence of an onocerane skeleton was demonstrated by the appearance of an olefinic methyl at δ 2.02 and an olefinic proton at δ 4.41. Thus, from the spectral data, compound **CSM1** was found to be onocer-7-ene-3α,21β-diol which was confirmed by comparing with the authentic compound.

When the methanol extract was eluted with chloroform-methanol (85:15), compound **CSM2** was isolated as an amorphous powder. It gave positive Mg-HCl and Molisch test indicating it to be a flavone. Its IR spectrum showed absorptions at 3379 cm^{-1} (hydroxy), 1655 cm^{-1} (carbonyl). The ^1H NMR spectrum of **CSM2** showed a very low field position of the OH proton singlet at δ 13.16 which confirmed the presence of a 5-OH group. The ^1H NMR spectrum exhibited singlets at δ 6.79 (1H) and 6.17 (1H) which could be assigned to H-3 and H-6, respectively. H-3 and H-6 with no proton neighbours appear as singlets at δ 6.79 and 6.17, respectively. Furthermore, the ^1H NMR spectrum of **CSM2** also exhibited two doublets at δ 8.03 (1H) and 6.92 (1H) which could be assigned to H-2′ (H-6′) and H-3′ (H-5′), respectively. *Ortho*-related H-2′ and H-3′ (and H-5′, H-6′) appear as doublets (*J* = 9.0 Hz) at δ 8.03 and 6.92, respectively. H-2′ and H-6′ are identical (i.e., signals superimposed) as also are H-3′ and H-5′. Glucosyl protons were observed at δ 3.76-3.86 (3H) and δ 4.57-4.92 (3H), combined signals integrate for 6H. The H-1 of glucose resonates at δ 5.03 (1H), which shows that D-glucose is α-linked (a broad singlet, *J* = 2.0 Hz). If it is β-linked, it

should have a large coupling constant (6.0 Hz) of H-1. The ^{13}C NMR spectrum of **CSM2** showed a signal at δ 182.04 due to carbonyl carbon. The presence of a C-linked at C-8 is evident from the shift of the C-8 signal to 104.53 p.p.m. (from its normal position of c. 94.00 p.p.m. in 5,7-dihydroxy flavonoids (Harborne and Mabry, 1975). Again, *C*-glucoside (8-linked) was evident from the presence of a signal at δ 73.30 due to C-1″ of the glucosyl group, otherwise it should be at around δ 100.00 due to C-1 resonances of *O*-glycosides.

The positive Fast Atomic Bombardment Mass Spectrum (FAB MS) showed a prominent peak at m/z 433 due to [M+1]. The mass spectrum exhibited a peak at m/z 415 [M-17] due to loss of hydroxyl radical ($OH^{\cdot}$). This usually involves internal ring formation and is commonly associated with 4′-hydroxylation in flavanoids (Harborne and Mabry, 1975). The fragmentation of compound **CSM2** shows fragments ion at m/z 314 due to A_1 and a base peak at m/z 313 due to [A_1-H]. It produces a peak at m/z 283 which consist of the aglycone fragment containing a CH_2 remnant of the original C-linked sugar. The molecular weight of this ion is useful in defining the aglycone type, which is found to be apigenin (m/z 270). Again, the aglycone, apigenin shows a [M-sugar-28] peak at m/z 242, which indicates loss of CO from the 4-keto function to form a five-membered ring. Thus, from these spectral data, it is found that compound **CSM2** is apigenin 8-*C*-α-D-glucopyranoside, adnatoside.

A grey-coloured residue was separated by further elution of the column with chloroform-methanol (85:15) and after purification, compound **CSM3** was isolated. The compound was found to be β-amyrin [olean-12(13)-en-3-one] whose structure was confirmed by comparison with an authentic sample.



CSM1 **CSM2** **CSM3**

Fig. 1. Chemical Structure of CSM1, CSM2 and CSM3.

Six compounds, including β-sitosterol and β-sitosterol-D-glucoside, were isolated from the methanol extract of *Smilax lanceaefolia* roots (Laitonjam and Kongbrailatpam, 2010) (figure 2). Compound **SL1** was obtained as colourless needles, mp 180-182^{o}C, IR (KBr, ν_{max}): 3387, 1643, 1379, 1296, 1070, 1032, 820, 772 cm^{-1}. The negative ion FAB-MS showed the quasi-molecular ion peak at m/z 449 [M-H] and its molecular formula was determined to be $C_{21}H_{22}O_{11}$ [(M-H) 449.1107 calculated as $C_{21}H_{22}O_{11}$, 449.1084] by high resolution FAB-MS. A positive ion FAB-mass spectrum of compound **SL1** exhibited pseudo-molecular ion at m/z 473 $[M+Na]^+$, thus confirming its M^+ to be 450 (calc. for $C_{21}H_{22}O_1$). The fragment ions at *m/z* 451 [M+H] and *m/z* 289 $[M+H– 162]^+$ indicated loss of the hexosyl moiety. The ^1H NMR spectra suggested that compound **SL1** was a flavonol and possessed a hexose. On acid hydrolysis, compound **SL1** afforded rhamnose that was identified as α-L-rhamnose by detailed analysis of NMR data. The ^1H NMR spectrum of **SL1** showed the presence of a methyl group at δ 1.20 (3H, d, J=6.3 Hz, H-6″).

Extensive analysis of the ^{1}H NMR data of **SL1** with the aid of ^{1}H-^{1}H COSY indicated that **SL1** possessed 5,7-disubstituted A-ring and 3$^{/}$,4$^{/}$-disubstituted B-ring. The ^{13}C NMR (DEPT) spectrum of **SL1** contained twenty one signals (C x 8, CH x 12, CH_3 x 1) that included a rhamnose moiety at δ 17.8 (CH_3), 70.5 (CH), 71.7 (CH), 72.1 (CH), 73.8 (CH), and 102.4 (C-1$^{//}$). Two oxygen-bearing methane protons assignable to 2 and 3 positions of the aglycone (δ_H 5.11, 1H, d, J=11.4 Hz, 2-H; 4.60, 1H, d, J=11.4 Hz, 3-H) showed a big J-value thereby revealing that 2,3 protons to be trans. Furthermore, the remarkable up field shifts of H-5$^{//}$ and H_3-6$^{//}$ of rhamnose due to the aromatic shielding effect contributed by B-ring, led us to conclude that the configurations at C-2 and C-3 to be 2S, 3S. Thus the structure of **SL1** was confirmed from the spectral data.

Compound **SL3** was obtained as a white amorphous powder. It gave a positive Liebermann-Burchard test and Molish reaction but not respond to the Ehrlich reagent. Compound **SL3** possessed broad absorption bands at3450 and 1045 cm^{-1} in its IR spectrum, indicating a glycosidic structure. The FAB mass spectrum gave quasi-molecular ion peaks at m/z 747 [M+Na] and 725 [M+H]. The molecular formula of **SL3** was estimated as $C_{39}H_{64}O_{12}$ by ^{13}C NMR and FAB mass spectrometry. Compound **SL3** was predicted to be a glycoside of a (25S)-spirostanol steroid based on the characteristic absorption bands at 990, 920, 900 and 850 cm^{-1}, with the absorption at 920 cm^{-1} being of greater intensity than at 900 cm^{-1} in the IR spectrum.

The ^{1}H NMR spectrum of **SL3** exhibited three secondary methyl proton signals at δ_H 1.64 (J=6.2 Hz), 1.15 (J=6.9 Hz) and 1.08 (J=7.1 Hz), two tertiary methyl proton signals at δ_H 0.81 and 0.67, and two anomeric proton signals at δ_H 5.53 (br s) and 5.00 (d, J=7.6 Hz). The signal at δ_H 1.64 was due to 6-deoxyhexose. The ^{13}C NMR spectrum showed 39 carbon resonances. The number of the attached hydrogens to each individual carbon atom was determined by the DEPT spectrum, which indicated the presence of 5 x Me, 12 x CH_2, 19 x CH, and 3 x C. The above data suggested that **SL3** was a (25S)-spirostanol disaccharide. On acid hydrolysis with 1M hydrochloric acid (dioxane-water, 1:1), **SL3** afforded D-glucose and L-rhamnose as sugar moieties identified by TLC with authentic samples, and a sapogenin, which was identified as (25S)-5α-spirostan-3β-ol (neotigogenin) by the IR and ^{1}H NMR spectra. The assignment of carbon signals due to the monosaccharides of **SL3** was carried out by comparison with the chemical shifts of methyl glycosides, and by considering the glycosylation shift. The ^{13}C NMR chemical shifts of a terminal α-L-rhamnopyranosyl unit could be attributed. The C-6 signal of the inner β-D-glucopyranosyl unit was observed at δ_C 68.6, indicating the involvement in glycosidic linkage formation. The anomeric proton signal of the glucose of **SL3** at 5.00 (d, J=7.6 Hz) showed that the glucosyl band of **SL3** had the β-configuration. However, on the basis of the coupling constant of the anomeric proton of rhamnose in the ^{1}H NMR spectrum, an α- or β- nature could not be deduced ; but α-rhamnose could be identified from its ^{13}C NMR chemical shifts, with C-5 of α- or β-rhamnose appearing at δ_C 69.8 and 73.5, respectively. The C-5 signal of the rhamnose unit in compound **SL3** was at δ_C 69.8, showing that rhamnose has the α-configuration. In the ^{13}C NMR spectrum of **SL3**, the chemical shift of C-3 at δ_C 69.8 77.7 revealed that the compound was the 3-*O*-glycoside. Thus, the structure of **SL3** was determined to be neotigogenin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

The IR spectrum of **SL2** showed the peak at 3398 cm^{-1} due to hydroxyl group**.** Its ^{1}H NMR spectrum showed the peaks at δ 0.79**,** 0.92**,** 0.96 and 1.02 due to methyl protons at H–18**,** H–27**,** H–21 and H–19**,** respectively**.** The hydroxyl proton at 3–position showed at δ 5.35**.** Its ^{13}C NMR spectrum showed at δ 16.259**,** 19.396**,** 14.499 & 17.107 due to methyl carbons at C–18**,** C–19**,** C–21 & C–27**,** respectively**.** Compound **SL2** was found to be the aglycone of **SL3**.

The structure of **SL4** was determined by its IR**,** ^{1}H NMR**,** ^{13}C NMR spectral data**.** Its IR showed peak at 3408 cm^{-1} due to hydroxyl group**.** Its ^{1}H NMR spectrum showed the peaks of methyl groups at δ_H 0.68**,** 0.82**,** 0.85**,** 0.93**,** 1.01 due to H–18**,** H–27 & 29**,** H-26**,** H- 21**,** H–19**,**

respectively. The olefinic protons at H–22 & H–23 showed the peaks at δ_H 5.14 & 5.05, respectively. The protons corresponding to sugar moiety showed the peaks at δ_H 4.39, 3.23, 3.58, 3.35, 3.76 and 3.80, respectively. The ^{13}C NMR spectrum of **SL4** showed at δ_C 10.8, 18.2, 17.7, 18.0, 10.9 due to methyl groups at C–8, C–19, C–21, C–26, C–27 & C–29, respectively. The olefinic carbon atoms at C–5, C–6, C–22 & C–23 showed the peaks at δ_C 139.3, 121.2, 137.3 & 128.9, respectively. The mass spectrum showed at m/z 397 due to [(M+1)–Glucose]. Thus, from all the above spectral data, the structure of compound, **SL4** was assigned as Stigmasterol glucoside which was confirmed by comparing the MS, ^1H and ^{13}C NMR spectral data with those reported in the literature and by taking the authentic sample.

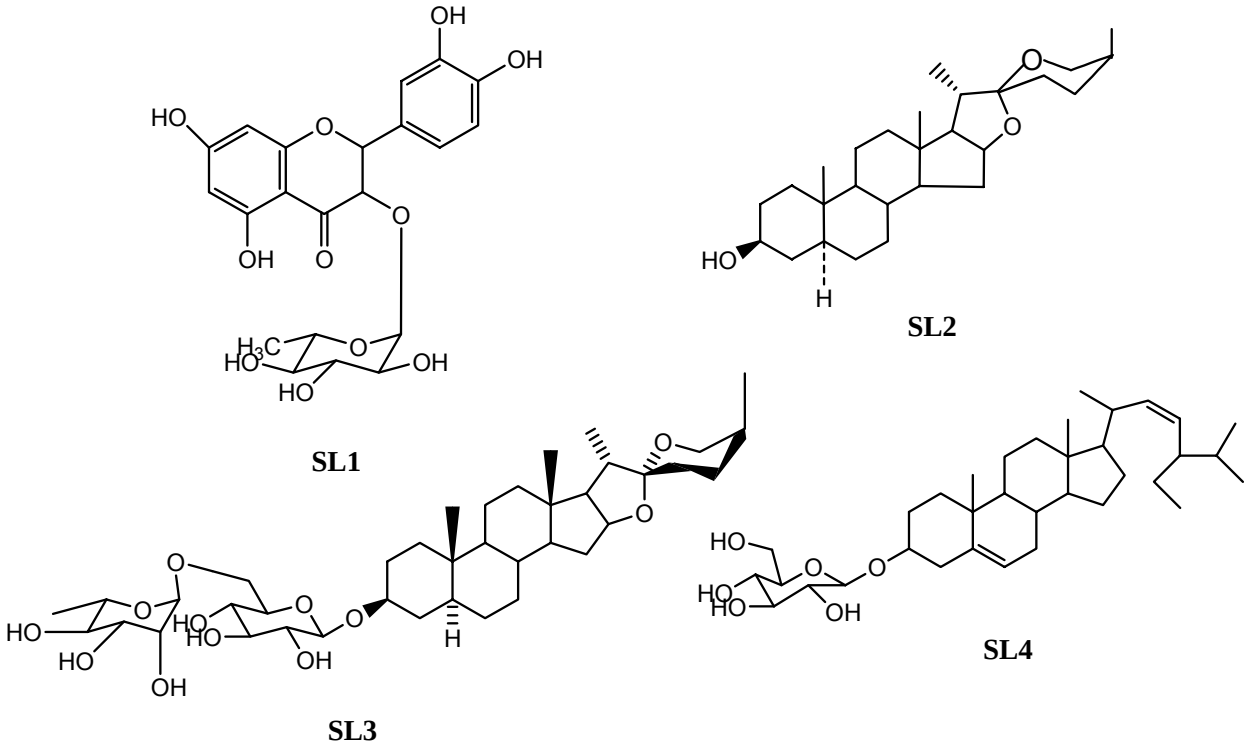


Fig. 2. Chemical Structure of SL1, SL2, SL3 and SL4.

3.2 COMPARISON OF CHEMICAL COMPOSITION

The moisture and mineral contents of *Smilax lanceaefolia* roots and that of *Cissus adnata* leaves are presented in Table 1. For comparison purposes, values of Yerbamate, Indian tea and black tea, according to some previous studies, are also included.

Moisture content formed the bulk of tissue weight in the fresh *Smilax lanceaefolia* roots and in the fresh *Cissus adnata* leaves with mean values of 41.83% and 87.28%, respectively. There is a high content of minerals such as iron (45.1 mg/100g sample), followed by magnesium (39.8 mg/100g sample) and calcium (24.7 mg/100g sample) in the roots of *Smilax lanceaefolia*. Similarly, in the leaves of *Cissus adnata*, there is also a high content of minerals such as calcium (299.0 mg/100g sample), followed by iron (56.2 mg/100g sample), magnesium (22.9 mg/100g sample) and manganese (22.9 mg/100g sample).

3.3 ANTIOXIDANT ACTIVITY

The total antioxidant activities of methanol extract (ME) of *Smilax lanceaefolia* roots, *Cissus adnata* leaves and Indian tea varieties – Assam tea and Darjeeling tea – were examined by DPPH free radical scavenging activity (Blossi, 1958) and β-Carotene-linoleate (Jayaprakasha et al., 2001) methods. The antioxidant activities of the compounds CSM1, CSM2, CSM3, SL1, SL2, SL3 and SL4 were also taken.

Table 1. Chemical composition of *Smilax lanceaefolia* roots, *Cissus adnata* leaves, Yerbamate, black tea and Indian tea

Analysis	*Smilax lanceaefolia* Roots[a]	*Cissus adnata* leaves[a]	Yerbamate[b]	Indian tea[b]	Black tea[c]
Moisture content (%)	41.83 ± 0.1	87.28± 0.1	9.00	9.3	3.9 - 9.5
Minerals (mg/100g)					
Calcium	24.7 ± 0.00	299.0 ± 0.07	664	426	-
Magnesium	39.8 ± 0.05	22.9 ± 0.02	-	-	-
Copper	3.1 ± 0.01	5.1 ± 0.02	-	-	-
Manganese	1.1 ± 0.00	29.8 ± 0.08	-	-	-
Iron	45.1 ± 0.06	56.2 ± 0.08	-	15.2	-
Cobalt	6.8 ± 0.005	8.0 ± 0.005	-	-	-
Zinc	1.3 ± 0.01	2.2 ± 0.06	-	-	-

Each value represents the mean ± SD of 6 determinations; [a]Concentration of element in mg/100g of the sample.
[b]Reference : Hart and Fisher (1971); [c]Reference: Pearson, (1976)

3.3.1 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method

The DPPH radical scavenging activities of methanol extract of *Smilax lanceaefolia* root (CR), of SL1, SL2, SL3, SL4, the methanol extract of Assam tea (AT) and of Darjeeling tea (DT) are presented in figure 3. CR of *Smilax lanceaefolia* root showed relatively high DPPH radical scavenging activity, with an average of 67.6%. The DPPH radical scavenging activity of SL1, SL2, SL3 and SL4 were found to be in the order of 34.88%, 2.33%, 2.30% and 47.44%, respectively. The DPPH free radical scavenging activity of methanol extracts of tea leaves of Assam tea (AT) and Darjeeling tea (DT) were 56.6%and 65.8%, respectively. The RSA (radical scavenging activities) are in the following order:

CR>DT>AT>SL4>SL1>SL2>SL3

The DPPH radical scavenging activity of methanol extract of *Smilax lanceaefolia* root which is used as herbal tea in North-East India is higher than that of the two varieties of Indian tea. Also among the isolated compounds, SL4 has highest radical scavenging activity.

Similarly, The DPPH radical scavenging activity of various extracts of *Cissus adnata* leaves was determined and the results are given in figure 4. It was found that the DPPH radical scavenging activity of active components from methanol extracts, CSM2 and CSM3, were found to be in the order of 5.40% and 1.44%, respectively. The DPPH radical scavenging activities of crude

methanol extract (ME) of *Cissus adnata* leaves showed relatively high DPPH radical scavenging activity, with an average of 52.88%. The DPPH radical scavenging activities of crude petroleum ether and chloroform extracts, PE and CL, were found to be in the order of 1.92% and 1.83%, respectively.

The RSA are in the following order:

ME>CL>CSM2>PE>CSM3

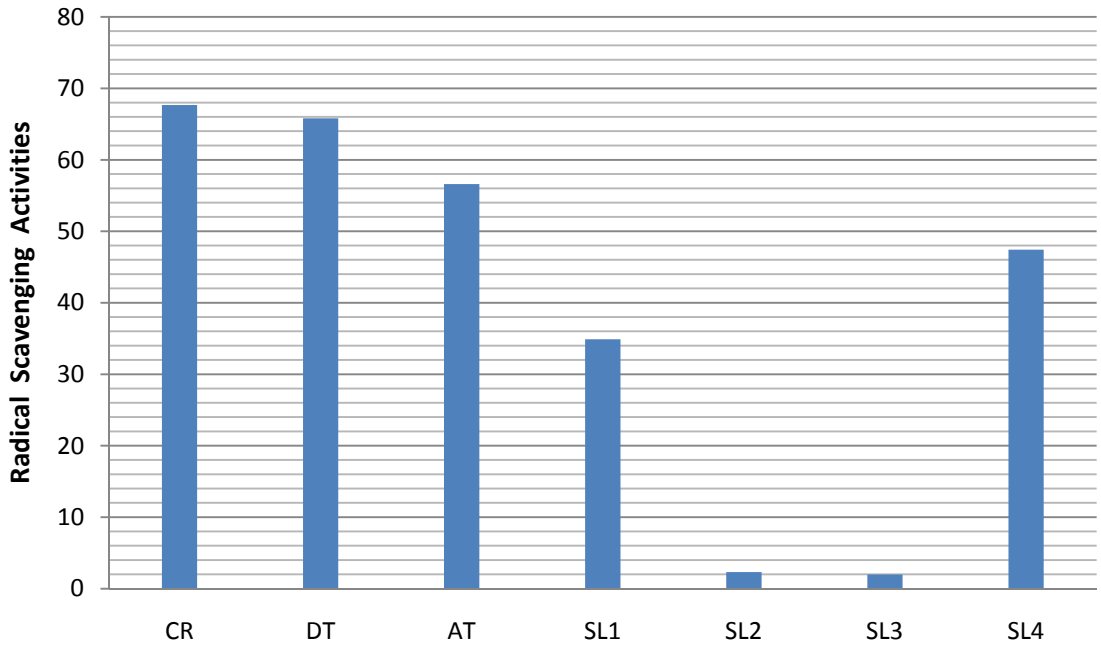


Figure 3. RSA of Methanol Extracts of *Smilax Lanceifolia* Roxb (CR), Darjeeling Tea (DT), Assam Tea (AT), and active components from Methanol extracts of *Smilax Lanceifolia* Roxb.

3.3.2 β-Carotene-linoleate method

Results of radical scavenging activities of *Smilax lanceaefolia* roots and *Cissus adnata* leaves using β-Carotene-linoleate method are shown in tables 2 and 3, respectively. It is to be noted that the methanol extract of *Smilax lanceaefolia* has highest absorbance value among the different extracts, even higher than that of BHT which is taken as a reference. Among the isolated compounds, SL4 has highest absorbance value. The absorbance values are in the following order:

M.E.> BHT >SL4 >SL3

For *Cissus adnata* also, methanol extract shows highest absorbance value followed by chloroform extract. The absorbance value of the methanol extract is even higher than that of BHT. The absorbance values follow the following order:

M.E.> CSM2 >CSM1 >CSM3 >BHT > CL

In the β-carotene method, the decrease in the concentration of β-carotene is measured indirectly by recording the absorbance value. As optical density or absorbance is directly proportional to

concentration, the higher the concentration of β-carotene the higher will be the absorbance value. Again, the high concentration of β-carotene corresponds to the high radical scavenging activity of the extract or compound as the active component protects β-carotene from the attack of linolic acid free radical to some extent by stabilizing the free radical.

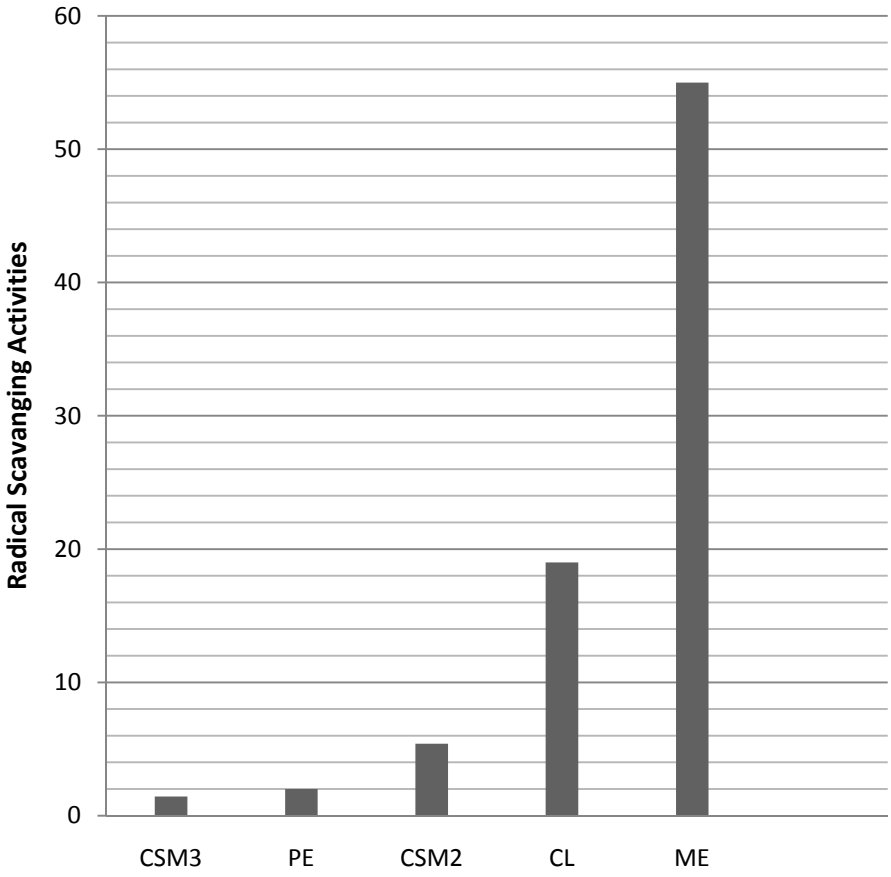


Figure 4: RSA of Petroleum ether extract (PE), Chloroform extract (CL), Methanol extract (ME), and active components from Methanol extract of *Cissus Adnata* Roxb.

Table 2. RSA of BHT, Petroleum extract (PE), Chloroform extract (CL), Methanol Extracts of *Smilax Lanceaefolia* Roxb (ME), and active components from methanol extracts of *Smilax lanceaefolia* Roxb. (β-Carotene-linoleate method)

Sl. No.	Code no.	Absorbance value
1	BHT	0.033
2	PE	-0.190
3	CL	-0.010
4	Me	0.161
5	SL1	-0.056

Table 3. RSA of BHT, Petroleum ether extract (PE), Chloroform extract (CL), Methanol extract (ME), and active components from petroleum ether and methanol extracts of *Cissus adnata* Roxb. (β-Carotene-linoleate method)

Sl. No.	Code no.	Absorbance value
1	BHT	0.033
2	PE	-0.019
3	CL	0.004
4	Me	0.145
5	CSM1	0.042
6	CSM2	0.051
7	CSM3	0.035

4. CONCLUSION

The isolation of active compounds and characterization of the structures of the isolated compounds from the plants, *Cissus adnata* Roxb. and *Smilax lanceaefolia* Roxb. were done using spectral data. The comparative study of the chemical components present and antioxidant activities of the two plants showed that the isolated compounds were found to have moderate to high free radical scavenging activities.

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