

Antioxidant and Cytotoxic activity of Steroidal Alkaloids Isolated from *Sarcococca saligna* against DPPH and HeLa Cell Lines

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ABSTRACT

Background and Objectives: Cancer is believed to be one of the main challenges to health care profession and compounds isolated from natural products play significant role in treatment of cancer. *Sarcococca saligna* (Buxaceae) contains variety of steroidal alkaloids having numerous pharmacological and biological potentials. Hence, current research work was designed to isolate steroidal alkaloids from *Sarcococca saligna* and to evaluate it for antioxidant and cytotoxic activity. **Materials and Methods:** Compounds Alkaloid-C, Dictyophlebine, Sarcovagine-D and Holaphylline were purified by column chromatography followed by spectroscopic techniques for their structure confirmation. Antioxidant and cytotoxic activities were investigated against DPPH and HeLa cell lines using ascorbic acid and doxorubicin as standard. **Results:** The radical scavenging effect produced by Holaphylline and Sarcovagine-D was 80.0 ± 3.20 and 78.0 ± 3.20 % at $400 \mu\text{g/ml}$ concentration which was statistically similar to that of standard, while on the same concentration inhibition for Dictyophlebine and Alkaloid-C was $10.0 \pm 0.00\%$ and 20 ± 1.01 %. In addition, Holaphylline showed less anticancer activity (23.88 ± 0.243) in cell growth of HeLa cell followed by Alkaloid-C (12.9 ± 0.235), Dictyophlebine (6.1 ± 0.345), while S cytotoxic effect for Sarcovagine-D was high (5.1 ± 0.14) in (IC_{50}), which exhibiting statistical variation from Doxorubicin ($2.1 \pm 0.14\%$). **Conclusion:** This study provided scientific report and importance of the use of steroidal alkaloids from *Sarcococca saligna* as an antioxidant and cytotoxic against agents' HeLa cell lines that may provide a new scope toward the development of novel drugs for cancer remedy.

Key words: *Sarcococca saligna*, Alkaloid-C, Dictyophlebine, Sarcovagine-D, Holaphylline, Doxorubicin, IC_{50} .

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INTRODUCTION

Natural products are important source of new chemical substances with potential therapeutic effects and are reported as a source of remedy for all ages people.¹ Researchers confirmed the role of microorganisms and secondary metabolites in the development of several allopathic drugs that are used as a therapeutics agents.¹ Additionally, several active compounds are synthesized from natural products that has historical importance in the development of several novel drugs.² Literature has shown that 80% (4 billion) of people

living in developing countries relies on plant based medicines in their health care management.^{3,4} Worldwide, 250,000 to 500,000 genera of natural products exists and only 1% are studied for pharmacological activities. Research is ongoing on plants for the isolation and identification of bioactive compounds rather than to investigates the whole characteristics.^{1,5} Approximately, 1572 genera and 5521 species of plants are present in Pakistan, out of which only 600 are used traditionally against various health conditions.⁶ A number of Pakistani



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plants are known to be beneficial in controlling excessive amount of free radicals and management of overproduction of cancerous cells. Plant based medicines has an excellent role in treatment of various diseases having minimum side effects as compared to allopathic medicines.⁷ Active compounds isolated from natural products has shown significant biological potential in treating various diseases. *Sarcococca saligna* is one of the members of Buxaceae family, found in northern region of Pakistan and are said to be an effective for treatment of various diseases.⁸ *Sarcococca saligna* was previously reported as a rich source of steroidal alkaloids that is responsible for various pharmacological and biological activities. Their extracts were scientifically reported for having significant properties against malaria, pain, fungi and microbes that caused various skin diseases.^{9,10} It is documented in literature that steroidal alkaloid of *Sarcococca saligna* has significant activities against diarrhoea, hypersecretion, leishmania, bacterial and potent acetylcholinesterase inhibitors effects.^{8,11} However, until now no study showed the antioxidant and anticancer potential of *Sarcococca saligna*. Hence, present research is design to isolate and evaluate the antioxidant and anticancer activity of the steroidal alkaloid isolated from *Sarcococca saligna*.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Sarcococca saligna (D. Don) Muel plant was collected from District Swat, Miandam in the month of June, 2014, Khyber Pakhtunkhwa, Pakistan. Authentication was performed by Dr. Jilani (botanist), with a specimen voucher (BUT.20098 (pup)) submitted to the herbarium section of botany department, University of Peshawar in Pakistan.

Chemicals and Instruments

Column chromatography technique, silica gel (SiO_2) and alumina (Al_2O_3) along with various reagents of different analytical grades were used. Visualization of alkaloids was done by TLC plates of Merck model number (GF-254). IR Jasco model (A-302), Hitachi UV spectrophotometer model (UV-3200), Mass spectrometer (Jeol H-X-110) and A-C-300 ^1H NMR were used for determination of the structure of the compounds.

Extraction and Fractionation of *Sarcococca saligna*

Collected *Sarcococca saligna* was washed with running water and placed under shade until dried and pulverized into powder (coarse) by mechanical blender. Crude powder was extracted by maceration technique using

hydro-methanolic mixture (8:2 ratios) in 35 L for a periods of two weeks, filtered and then concentrated under vacuum (2 kg).¹² After collection, to concentrated extract ($\text{MeOH}/\text{H}_2\text{O}$), 2 L of D/W was poured and extracted using different solvent system based on polarity e.g. n-hexane, chloroform, ethyl acetate and butanol for collecting different fractions i.e. butanol (100 g), ethyl acetate (150 g), chloroform (200g) and n-hexane (254 g).¹²

Identification of compounds by Colum and thin layer chromatography

After performing pilot study on each fraction against cancer cells, fraction of chloroform was selected on the basis of higher efficacy and was subjected for e isolation of alkaloids by spraying dragendroff's reagent. Chloroform extract was further fractionated by neutral alumina (Al_2O_3) and silica gel using a few drops of diethylamine alongside by different ratio of n-hexane and ethyl acetate mixture for purification of compounds as reported previously in literature.¹³ The sample that was collected from column fractions were investigated on TLC plate by using suitable solvent system to find the retention factor (R_f) that appeared as spot. Fraction with equal R_f values were combined. Results on TLC plates indicate the presence of alkaloids in chloroform fraction. For isolation of purified compounds, various sub-fractions were subjected again to column chromatography using small glass column with suitable solvent system. Fractions F1-F3 were collected from chloroform fraction by using a few drops of diethylamine with a mixture of n-hexane and ethyl-acetate solvent of different ratio through column chromatography.

Structure Elucidation of Isolated Compounds

Two compounds "Alkaloid-C and Dictyophlebine" were obtained by re-fractionation of F1 with solvent ratio 90:10, while Sarcovagine-D with solvent ratio 80:20 from fraction F2 and Holaphyline from F3 with a ratio 75:25 through thin layer chromatography.

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectrometry was carry out by using Spectrometer model (JEOL JNM-ECA 500), as described by researcher previously. The ^1H and ^{13}C spectra were recorded at wavelength of 500.00 and 125.00 MHz, respectively. Sample was diluted in 0.8 mL Chloroform deuterated (CDCl_3) and placed into NMR tube for making sample depth around 3.5cm^{-1} to 4cm^{-1} and ready to be analysed by NMR spectrometer.¹⁴ Chemical shifts were recorded as δ units (ppm) with tetramethylsilane (TMS) as internal standard and coupling constants (J) in Hz. Integration of the ^1H -NMR and

^{13}C -NMR data was performed by using DELTA version 5.0.4 software by JEOL. Identification of ^1H -NMR and ^{13}C -NMR was based on NMR absorptions that were published in Organic Chemistry,¹⁵ and possible proposed structure given by NIST library.

Alkaloid-C

Alkaloid-C was isolated from the chloroform fraction of *Sarcoca saligna* by subsequent elution of sub-fraction NF23-4 on neutral alumina column with solvent increasing polarities of EtOAc / n-hexane with a few drops of diethylamine. It appear in form of a white amorphous powder (Figure 1), with M.P= 155°C (Reported value 152-153°C), $[\alpha]_{25}^{\text{D}}: -30^\circ$ (0.040, CHCl_3), UV (MeOH)- nm (log ϵ): λ_{max} : 239.0 (2.330), IR (CHCl_3) $\nu_{\text{max}}^{\text{cm}^{-1}}$: 2931.0 (= CH), 511($\text{C}=\text{CH}$), EI MS m/z (rel.int.%): 359.0 (M+,11.0), 44 (36),72 (100g). FAB+ve MS; m/z 360. HREI MS m/z : 359.3225 (calcd for $\text{C}_{24}\text{H}_{41}\text{NO}$, 359.3226). ^1H -NMR, δ (400 MHz, CDCl_3): 0.65 (3 H, s, CH_3 -18), 0.98 (3 H, s CH_3 -19), 0.860 (3 H, d, $J_{21,20} = 6.40$ Hz, CH_3 -21), 2.140 (6 H, s, H-N CH_2), 2.42 (1-H, m, H-20), 3.04 (1 H, m, H-3), 3.330 (3 H, s, OCH $_3$), 5.34 (1-H, d, H-6).

The optical rotation $[\alpha]_{25}^{\text{D}}: -30^\circ$ (c 0.04, CHCl_3) indicated the presence of chiral centres, while the UV spectrum displayed absorption at 239nm. The HREI MS of compound showed the $[\text{M}^+]$ at m/z 359.3125 (calcd for $\text{C}_{24}\text{H}_{41}\text{NO}$, 359.3126). The ^1H -NMR displayed two up-field singlets at δ 0.65 and 0.98, having properties of C- $_{18}$ and C-19 angular methyls. A C-21 secondary methyl was showed at δ 0.86 doublets ($J_{21, 20} = 5.0$ Hz), while signal at δ 2.14 for 6H singlet was ascribed to NMe $_2$ protons. A C-20 methine proton was appeared at δ 2.42 respectively while multiple at δ 3.04 was assigned to the C-3 methine proton. A methoxy proton was present δ 3.33 of a 3H singlet. At δ 5.34 downfield signals was present due to the C-6 methane proton. ^{13}C -NMR spectra of Alkaloid-C demonstrated total of 24 carbons, which contained six methyl, eight methylene, seven methine and three quaternary carbons. The spectral data showed that compound Alkaloid C was first reported from the same species *Sarcococca saligna*.¹⁶ The Structure, Mass spectra and ^1H NMR of Alkaloid C are presented in Figure 1, 2 and 3.

Dictyophlebine

Dictyophlebine was isolated from chloroform fraction of *Sarcococca saligna* by repeated elution of sub-fraction NBEA on neutral alumina column with increasing solvent polarities of n-hexane/ EtOAc and a few drops of diethylamine. Dictyophlebine appear in form of a white amorphous powder with M.P (148)°C, (Reported

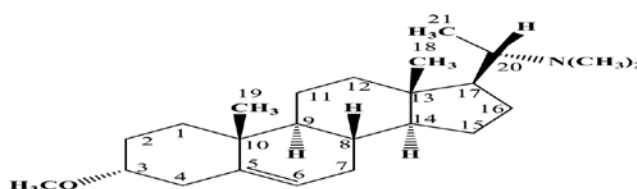


Figure 1: Structure of Alkaloid-C.



Figure 2: Mass spectra of Alkaloids-C.

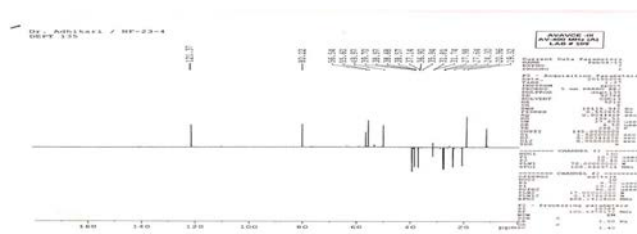


Figure 3: ^1H NMR of Alkaloid-C.

value 152-153°C). IR (CHCl_3), $\nu_{\text{max}}^{\text{cm}^{-1}}$: 3352.00 (N-H), 2925.0 (C-H). EI MS m/z (rel.int.,%): 360 (M+, 3), 345(8.), 289 (4),72 (100). FAB+ve MS: m/z 361. HREI MS m/z : 360.3029 (calcd for $\text{C}_{24}\text{H}_{44}\text{N}_2$, 360.3134). ^1H -NMR, δ (300.0 MHz, CDCl_3): 0.610 (3H, s, H-18), 0.750 (3H, s, H-19), 0.830 (3H, d, $J_{21, 20} = 6.40$ Hz, H-21), 2.130 (6H, s, N- CH_2), 2.390 (3H, s, N- CH_3). IR spectra of Dictyophlebine showed absorption at 3352 (NH), 2925 (CH) cm^{-1} while the HREI MS displayed the $[\text{M}^+]$ at m/z 360.3029 ($\text{C}_{24}\text{H}_{44}\text{N}_2$, calcd 360.3034). The ^1H -NMR showed signal of two up-field 3H singles at δ 0.61 and 0.75, for protons of C-18 and C-19 angular methyl's. The C-21 secondary methyl protons were appeared at δ 0.83 (3H, d, $J_{21, 20} = 6.4\text{Hz}$). The N $_b$ -Me $_2$ protons was at δ 2.13, while N $_a$ -Me $_2$ protons was resonate at δ 2.39 as 3H singlet. The ^{13}C -NMR spectra of Dictyophlebine demonstrate resonance of all 24 carbons which also include six methyl, nine methylene, seven methine and two quaternary carbons. The spectral characteristics of compound showed that it was Dictyophlebine, which was previously isolated from

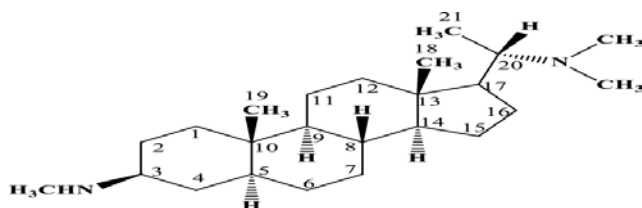


Figure 4: Structure of Dictyophlebine.



Figure 5: Mass spectra of Dictyophlebine.

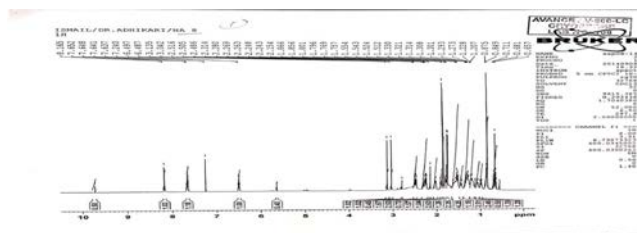


Figure 6: ¹H NMR of Dictyophlebine.

Dictyophlebia lucidia, *Funtumia latifolia*, *Sarcococca saligna* and *S. hookeriana*.¹⁷ Structure, Mass spectra and ¹H NMR of Dictyophlebine are shown in Figure 4, 5 and 6.

Sarcovagine-D

Sarcovagine-D was isolated from the chloroform fraction of *Sarcococca saligna* by repeated elution of sub-fraction NA on neutral alumina column with increasing solvent polarities of EtOAc / n-hexane and a few drops of diethylamine. It occurs as a solid white crystalline powder that has M.P= 173°C (Reported 170-172°C). EIMS m/z: 440(3), 425(5), 98(6), 83(44), 72(100), 58(4), 55(25). HREI- MS m/z: 440.3391, (Calcd - for C₂₈H₄₄N₂O, 440.3398). ¹H NMR, δ (400.0 MHz., CDCl₃): 0.69 (3H, s, H-18), 0.85 (3-H,s, H-19), 1.22(3-H,d, J_{21,20}=6.4 Hz,H-21), 1.78 (3H,d., J- 4., 3= 6.3 Hz, H-4'), 1.86 (3H,s, H-5'), 2.42 (6H,s, N-Me₂), 6.48(1H,quartet, J 3', 4'=6.6 Hz, H-3'), 7.66 (1H,dd,J_{2 1α} = 6.7 Hz, J_{2 1β} = 2.4 Hz, H-2), 8.26 (1H, s,- H-N). The optical rotation of Sarcovagine-D was [α]_D²⁵ = 29° (C = 0.03, CHCl₃), while the UV spectrum showed absorption at 212nm. The IR spectrum exposed absorption at 3398 (NH), 2927 (CH), 1660 (amidic C=O), 1630 (C=C)

cm⁻¹. The HREI MS of compound displayed the [M⁺] at m/z 440.3391 (Calcd for C₂₈H₄₄N₂O, 440.3398). The ¹H-NMR of compound 3 showed spectrum of three up-field singlets at δ 0.65, 0.86 and 1.87 were assigned to C-₁₈, C-19 and C- 5' tertiary methyls. The C-21 and C- 4' methyl protons were position at doublets δ 1.22 (J_{21,20} = 6.4 Hz) and 1.78 (J 4', 3' = 6.3 Hz), while the N-Me₂ protons were position at 6H singlet of δ 2.48, respectively. The C-2' and C-2 olefinic protons were position at two downfield signals of δ 6.48 as quartet (J 3', 4' = 6.6 Hz) and 7.64 as double doublet (J_{2 1α} = 6.7 Hz, J_{2 1β} = 2.4 Hz) respectively. The ¹³C-NMR spectra of compound 3 demonstrate resonance of all 28 carbons which include seven methyl, seven methylene, eight methine and six quaternary carbons. The spectra indicate that Sarcovagine-D compound is

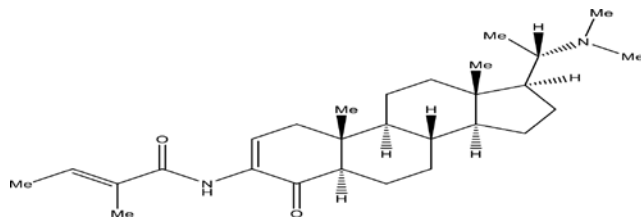


Figure 7: Structure of Sarcovagine-D.

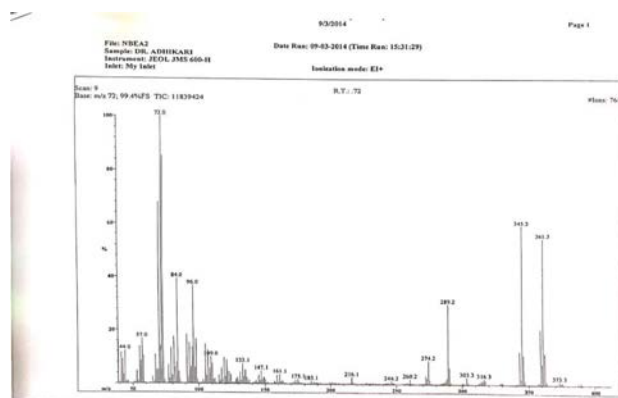


Figure 8: Mass spectra of Sarcovagine-D.

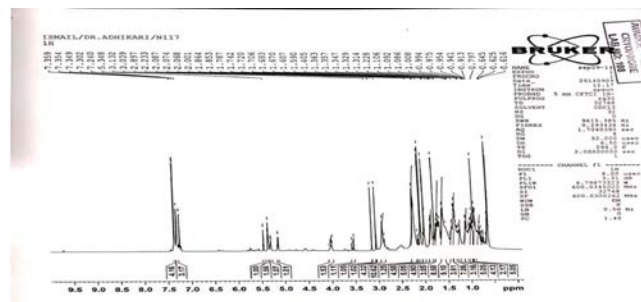


Figure 9: ¹H NMR of Sarcovagine-D.

well known and previously isolated and identified from the *Sarcococca vagans*.¹⁸ The Structure, Mass spectra and ¹H-NMR of Sarcovagine-D are shown in Figure 7, 8 and 9.

Holaphylline

Holaphylline was isolated from the chloroform fractions of *Sarcococca saligna* by subjecting on alumina column chromatography, increasing polarities of repeated elution of n-hexane/ EtOAc and few drops of diethylamine. It appear as sticky yellowish powder having M.P.=125°C (Reported 128°C). FD-MS: m/z 329.20 (C₂₂H₃₅NO). HREI-MS- m/z: 329.550 (calcd, C₂₂H₃₅NO, 329.4529). ¹H-NMR-(CDCl₃): δ 0.900 (3H, s, -CH₃-18), 1.000 (3H, s, CH₃-19), 2.100 (3H, s, CH₃-21), 2.622 (3H, s, CH₃-22), 2.990 (1 H, dd, H-3), 5.340 (1H, brs, H-6), 2.78 (1H, brs, H-17). ¹³C-NMR (CDCl₃); δ = 35.00 (C-1), 25.90(C-2), 59.20(C-3), 37.0(C-4), 137.70(C-5), 123.60(C-6), 31.90(C-7), 31.8(C-8), 49.30(C-9), 36.70(C-10), 20.9(C-11), 34.90(C-12), 45.40(C-13), 50.40(C-14), 24.30 (C-15), 25.10 (C-16), 61.20 (C-17), 20.60 (C-18), 19.20 (C-19), 212.70 (C-20), 32.80 (C-21), 29.90 (C-22). HR-ESI-MS showed pseudo molecular ion peaks [M+H] at m/z 330 corresponding to the molecular formula C₂₂H₃₅NO [Calculated as C₂₂H₃₅NO +H = 330.2797]. The IR spectrum indicated the presence of an amide carbonyl (1730 cm⁻¹). ¹H-NMR (CD₃Cl, 400 MHz) showed the signals of 3 protons each at δ 0.90, 1.0, 2.10 and 2.62 correspond to three CH₃-18, CH₃-19, CH₃-21 and CH₃-N. The ¹³C-NMR showed 22 signals correspond to 22 carbons skeleton of the compound, showing 3 quaternary carbons at δ 137.70, 36.70 and 45.40 while 1 carbonyl carbons at δ 212.70. The 2D HSQC presented 18 protonated carbons. The 2D (1H-1H COSY and HMBC) disclosed many of the correlations of the elucidated Structure. Data represent that the compound holaphylline first time isolated from *Sarcococca saligna* and previously isolated from plant *Holarrhena floribunda*.¹⁹ Structure, Mass spectra and ¹H-NMR of Holaphylline are shown in Figure 10, 11 and 12.

Antioxidant Activity of Compounds Isolated from *Sarcococca saligna*

Antioxidant activity of isolated compounds of *Sarcococca saligna* was investigated using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging method with slight changes in procedure by using UV spectrophotometers at 517 nm wavelength.¹³ In DMSO, various test compounds solution was prepared by diluting in 95µl of DPPH ethanolic solution. Solutions were dispensed into 96 well microplates and kept at room temperature (37°C) for a period of half-hour. The mean absorbance of the resulting solutions was obtained by

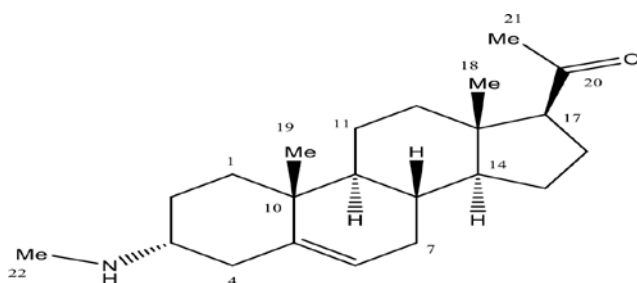


Figure 10: Structure of Holaphylline.



Figure 11: Mass spectra of Holaphylline.

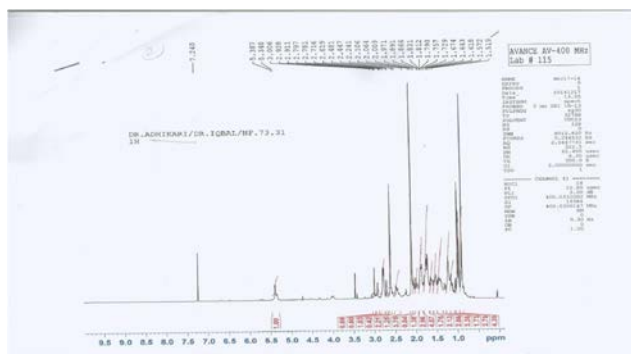


Figure 12: ¹H-NMR of Holaphylline.

spectrophotometers at a wavelength of 517 nm from triple reading of all samples after an incubation period of 1 hr in the dark. Vitamin C (Ascorbic acid) was used as an antioxidant standard. Measurements of both test and the blank solution were run in triplicate. The radical scavenging ability for compounds was calculated by comparing the test solution with ascorbic acid.

The formula used to measure radical scavenging as presented below;

$$\text{RSA (\%)} = 100 - (\text{Optical Density test sample} / \text{Optical Density standard sample}) \times 100$$

Cytotoxic Activity of Compounds Isolated from *Sarcococca saligna*

Cytotoxic activity of compounds isolated from *Sarcococca saligna* was determined by MTT standard colorimetric method with slight changes in the procedure.²⁰ MTT

(3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric technique with 96-micro well plates was used to assess cell viability. Dulbecco's eagles medium was used to culture HeLa cell after some modification by adding 10% fetal bovine serum (FBS) at 37°C along with 5% CO₂, 100 IU/mL penicillin and 200 µg/mL streptomycin in separate flasks in triplicates. In order to count the number of cells, cell lines were cultivated in a selected dilution medium and haemocytometer. 100 ml of microliters of cell culture at a density of 1 × 10⁵ cells/mL was prepared per well and transferred into 96-well microplate. Afterwards, plates were incubated overnight before replacing the medium with 200 µL of new freshly prepared medium at different concentration of 0.5 to 10 µM. 2 µg/mL was added to each well and incubated, subsequently, 100 µL of DMSO was added and kept for 48 hr and the absorbance was measured using ELISA plate reader at a wavelength of 570 nm.

Statistical Analysis

Collected data were expressed as mean ± standard deviation. Results were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Present research showed the biological activity of four compounds isolated from *Sarcococca saligna* against free radicals and cancer using DPPH and HeLa cell line model. By comparing the free radical scavenging capacity of the compounds and the growth inhibition of HeLa cell, a positive linear relationship between the antioxidant and anticancer was observed, suggesting that the antioxidant potentials of these compounds might contribute to their anticancer effect.

Antioxidant Activity of Compounds Isolated from *Sarcococca saligna*

Isolated steroidal compounds of *Sarcococca saligna* was tested for antioxidant activity using DPPH technique (Ascorbic acid as a standard). Results are shown in Table 1. All compounds produced a dose-dependent free radical scavenging activity while the scavenging activity showed by Sarcovagine-D and Holaphylline was significant by increasing compounds concentration 100 to 400 µg/mL. Radical scavenging activities of Sarcovagine-D and Holaphylline at concentration 400 µg/mL were 78% ± 0.01 and 80 ± 3.20%, respectively. However, antioxidant activity produced by Alkaloid-C and Dictyophlebine at 400 µg/mL was 20% ± 1.01 and 10% ± 3.40, respectively, which was low as compared to Sarcovagine-D and Holaphylline. The

relative potency of the isolated steroidal compounds was as follows: Sarcovagine-D > Holaphylline > Alkaloid-C > Dictyophlebine. Several research articles documented the beneficial role of steroidal alkaloids in scavenging of excessive generation of free radicals in body.²¹ Results of this study were therefore consistent with previous findings that *Sarcococca saligna* also contains a high quantity of steroidal compounds that possess promising scavenging effect in DPPH method. The free radical scavenging activity of *Sarcococca saligna* along with other isolated steroids has not been reported, despite its earlier description in the leaves of *Sarcococca coriacea*.²² Data were expressed as mean ± standard deviation, where experiments and measurements were carried out in triplicate for each group. % radical scavenging was determined from nonlinear regression using Graph Pad prism software. A value of $P < 0.05$ was considered statistically significant.

Cytotoxic Activity of Steroid alkaloid Isolated from *Sarcococca saligna*

Cytotoxic potential of isolated compounds was tested on HeLa cell lines (cervical cancer cell line) and was expressed as the percentage growth inhibition. Table 2 shows the IC₅₀ value of isolated compounds on HeLa

Table 1: Antioxidant activity of Steroidal alkaloid from *Sarcococca saligna*.

Tested Compound	Different Conc. in µg/ml	% Radical Scavenging Activity
Holaphylline	100	20±4.10
Holaphylline	200	35±3.00
Holaphylline	300	60±0.001
Holaphylline	400	80±3.20
Sarcovagine-D	100	15±2.10
Sarcovagine-D	200	35±1.00
Sarcovagine-D	300	55±3.03
Sarcovagine-D	400	78±0.01
Alkaloid-C	100	--
Alkaloid-C	200	--
Alkaloid-C	300	10±0.00
Alkaloid-C	400	20±1.01
Dictyophlebine	100	--
Dictyophlebine	200	---
Dictyophlebine	300	----
Dictyophlebine	400	10±3.40
Ascorbic acid	100	45±1.90
Ascorbic acid	200	65±0.001
Ascorbic acid	300	80±6.05
Ascorbic acid	400	95±.001

Table 2: Cytotoxicity steroidal alkaloids of *Sarcococca saligna* against HeLa cells.

S/No	Compounds	IC ₅₀ ±SD (µg/ml)
1	Alkaloid C	12.9±0.2
2	Dictyophlebine	6.1±0.3
3	Holaphylline	23.8±0.2
4	Doxorubicin	2.1±0.1
5	Sarcovagine D	18.1±0.1

cell lines. Results have shown that compounds isolated from *Sarcococca saligna* inhibit the cell proliferation *in vitro* as efficiently as the standard (doxorubicin). Table 2 documented that Dictyophlebine was more effective against cell proliferation as compared to other compounds, while Alkaloid-C exhibited moderate cytotoxic activity with IC₅₀ value of 12.98 ± 0.235 µg/mL followed by Sarcovagine-D with IC₅₀ value of 18.10 ± 0.14 µg/mL against HeLa cells. Holaphylline showed a very low inhibition in cell proliferation with IC₅₀ value of 23.88 ± 0.243 µg/mL as compared to other compounds and doxorubicin. This is compatible with reports from other works on other *Sarcococca saligna* alkaloids; sarsaligenines A and B that selectively suppressed the replication of human promyelocytic leukemia cells (HL-60), producing IC₅₀ values of 1.8 µg/mL and 2.3 µg/mL, respectively.²³

Moreover, Pregnane alkaloids isolated from *Sarcococca ruscifolia*, another species of *Sarcococca*, was reported to have cytotoxic effect on cancer cells.²⁴ Positive relationship between antioxidant and anticancer activity of these compounds was reported previously in literature,²⁵ suggesting that *Sarcococca* alkaloids can be used as a cytotoxic agent against cancer cells in future. The presence of other compounds in *Sarcococca saligna* such as sarcocucinine, sarsaligates A and B and Sarcovagine has documented, which have anticancer property in preclinical testing.²⁶ The cytotoxic and anti-proliferative effects produced by compounds isolated from *Sarcococca saligna* may be attributed to cell cycle arrest and apoptosis mechanisms as was previously reported by Badmus *et al.* in research article.²⁷ Additionally, the cytotoxic activity of steroidal alkaloids against cancer cells by inhibiting the cell growth was also documented in literature confirmed present study.²⁸ As previously reported in other studies that compounds showing cytotoxicity are beneficial in the treatment of chronic diseases, *Sarcococca saligna* may be used in the treatment of various chronic diseases. In addition to the above mechanism, several researchers confirmed that the cytotoxic activity of steroidal compounds may be

attributed to chemoprevention by signal transduction and angiogenesis.²⁸

CONCLUSION

Current research provides the proofs that steroidal alkaloid isolated from *Sarcococca saligna* has potent antioxidants properties that correlate with its cytotoxic effect on HeLa cell line. However, further studies are required to address the exact mode of action of these isolated compounds that can open the way to develop novel drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

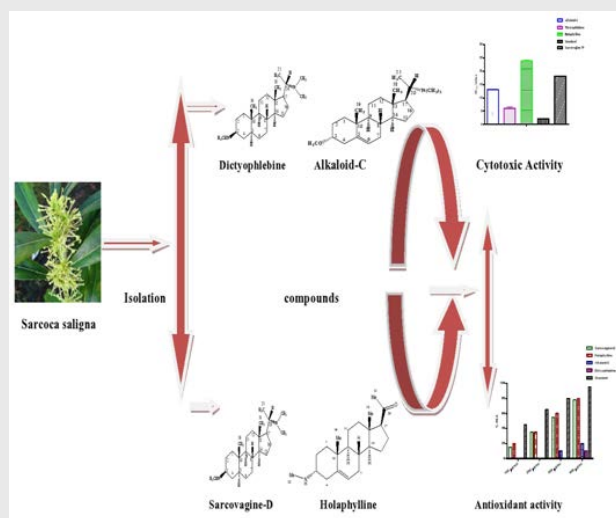
IC₅₀: Half Maximal Inhibitory Concentration; **µg**: Microgram; **NMR**: Nuclear Magnetic Resonance; **TLC**: Thin Layer Chromatography; **F1**: Fraction 1; **F3**: Fraction 3; **SD**: Standard deviation, **D/W**: Distilled water; **DPPH**: 1, 1-diphenyl-2-picrylhydrazyl.

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PICTORIAL ABSTRACT



SUMMARY

Currently, one of the main problems to health care profession is to find the safe and effective therapy for cancer. Compounds isolated from natural products play a significant role in treatment of various diseases. Hence, in present research work, four steroidal alkaloids were isolated from *Sarcococca saligna* and were evaluated for antioxidant and cytotoxic activity using DPPH and HeLa cell lines method. Ascorbic acid and doxorubicin was used as a standard. Radical scavenging activity of Holaphylline and Sarcovagine-D were statistically same to ascorbic acid, while low for Dietyophlebine and Alkaloid-C. Furthermore, anticancer activity of Holaphylline followed by Alkaloid-C, Dietyophlebine was low as compared to Sarcovagine-D against HeLa cell lines. This study serves as a proof for isolation of steroidal alkaloids from *Sarcococca saligna*, which might be responsible for treating excessive free radicals and cancer cells. More analysis will be highly appreciated to understand the exact mechanism(s) behind antioxidant and cytotoxic activity.

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