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Environmental Factors Affect Altitude on Coumarin Metabolites Accumulation, Docking Studies, Antiproliferative Activity on Indian Folklore Medicinal Plant Chloroxylon Swietenia Dc

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ABSTRACT

In natural plant species, the abiotic parameter height has been shown to influence secondary metabolite elaboration. To back up this theory, comprehensive chemical and biological research on the leaves of *Chloroxylon swietenia*DC, a strong Indian traditional medicinal plant, have been conducted. C. swietenia leaf samples were gathered at two dist inct elevations. *C. swietenia* DC leaves were obtained from the Jaipur woodland region (altitude: 159 meters), The furano-coumarin metabolites or their precursors were found exclusively in the state of Telangana. The pyarano-coumarin metabolite or its precursors were found exclusively in leaves taken from the Gadchiroli forest area (altitude: 217 metres) in Maharashtra state. Suberosin analogues and racemosin from the Gadchiroli area are more active than those from the Jaipur area. Because both furano-coumarins and pyrano-coumarins have been shown to have anticancer activity in the literature, the isolated compounds of C. swietenia was subjected to antiproliferative screening against some human cancer cell lines in order to identify potent lead compounds, as well as docking studies, i.e. 5ZSY (lung cancer) (-6.6).

KEYWORDS: Chloroxylon swietenia, Altitude, Pyrano-coumarins, Furano-coumarins, Anti proliferative activity, Docking studies.

INTRODUCTION

Plants produce two types of metabolites such as primary and secondary metabolites. They protect plants against stress [1, 2], both biotic and abiotic. When plants are subjected to biotic and abiotic stress, secondary metabolite synthesis normally increases. Different types of biotic and abiotic stress cause changes in morphological characteristics such as height, leaf number, leaf area, branch number, root volume [3] and so on. Nematodes, bacteria, viruses, and fungi [4] are among the living creatures that produce biotic stress in plants. The concentration of SMs drops dramatically during stress recovery [5].The crucial factors governing the altitudinal variation in the field has not been thoroughly investigated so far [6]. In general, plant species diversity declines with altitude [7]. Plant secondary metabolite altitudinal variation is similar to latitudinal variation. There are two main theories, both of which are partially contradictory. LupinusargenteusPursh populations found at higher elevations had much lower levels of poisonous quinolizidine alkaloids in their leaves [8]. Maxon and *Pteridium caudatum* (L). Maxon, which contained higher amounts of photo-7 protective and radical scavenging phenolics in higher altitude sites than in lower growing stands [9] the overall balance was loss of phytodiversity relative to larger areas found in lower elevations. The higher the altitude and the lower the latitude, the greater the gradient [10], If it is true that species richness generally declines with altitude, total alkaloid accumulation generally decreases with altitude. Molecular damage (UV energy reaching the ground, even at low altitude, may be quite damaging [11]. Acquired photo-energy can also be relaxed as heat; and absorption to promote chemical reactions. A classical example for the effect of altitude on the secondary metabolite accumulation is that of Zidorn [12], who has worked on the extraction and HPLC-based analysis of blooming heads collected at various elevations ranging from 180 to 1,060 m (Crepiscapillaris), 190 to 1,290 m (Hieraciumpilosella), and 20 to 1,290 m (Crepiscapillaris)(Hypochaerisradicata) There was a favourable correlation between the altitude of the growth site and the amount of flavonoids and phenolic acids in all of the taxa investigated.It is clearly seen that high altitude plants have developed highly specialized traits to withstand high levels of UV-B solar radiation, which may be necessary to elaborate diverse secondary metabolites. India is one of the 17 mega bio-diverse countries of the world with 15 agro climatic zones, 10 bio-geographic zones, 45000 different plant species, 17000 ~ 18000 flowering plants, 6000 ~ 7000 medicinal plants, 2400 plant species in codified ISM, 960 plants are in current trade. India is one of the 17 mega bio-diverse countries of the world with 15 agro climatic zones, 10 bio-geographic zones, 45000 different plant species, 17000 ~ 18000 flowering plants, 6000 ~ 7000 medicinal plants, 2400 plant species in codified ISM, 960 plants are in

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current trade [13]. As far as medicinal plant diversity in India is concerned, about 25~30% species grown in high altitudes. Till date very few high altitude plant species of India could be exploited chemically and biologically. Still a large number of these plants remain to be explored. As these plants grow at high altitude and at low temperatures, they are expected to elaborate diverse biologically active natural products. Recently *ChloroxylonswieteniaDC* a highly potent Indian folklore medicinal plant has attracted the attention of chemists and biologists due to its widespread accumulation in different geographical regions of India. It is of interest now to see the effect of various growth parameters, especially altitude on its phytochemical constituents and biological activities. In this connection, detailed literature search has been made on botanical, traditional, chemical and pharmacological properties of *C. swietenia* and presented the brief review below.*Chloroxylonswietenia*DC belonging toFamily Rutaceae, popularly known as Yellow wood, East Indian satin wood, Ceylon satin wood, is native to India and Sri Lanka [14]. In India it is commonly known as Bhirra (Hindi), Bhillotaka (Sanskrit), Billudu (Telugu), Vaaimaram (Tamil) [15]. The leaflets (10 - 20 pairs) are sub-opposite or alternate, oblong, obtuse, glabrous and glaucous. Flowers are white or cream in colour and present in terminal or axillary panicles. Flowers appear during March-April, fruits produce seeds [16].*C. swietenia* is considered as a potent medicinal plant having several medicinal uses in the folklore remedies [17]. The Malasar tribes of Tamil Nadu, South India apply the leaf paste on wounds, cuts, burns and skin diseases for quick recovery [18].The stem bark is credited for its effectiveness in the treatment of common cold and cough [19], ophthalmic infection & cataract, wounds [20,21]and as an stringent [22]. The various phytochemical constituents reported [23] from different plant parts of C. *swietenia.* The chloroform extract of the leaves exhibited significant anti-inflammatory response at various doses of 50, 100 and 200 mg/kg, when administered orally [24]. The ethanolic extract of the whole plant was reported to possess hepatoprotective activity at a dose of 25 mg/kg administered by oral gavage [25]. The secondary metabolites of *C. swietenia* crude extract and its fractions, such as hexane and n-butanol fractions showed good tyrosinase inhibition activity [26]. However,the variation in Phytochemistry and biological activities of *Chloroxylonswietenia* DC leaf growing in different altitudes have not yet been reported.

MATERIALS AND METHODS

General experimental procedure:

Melting points were taken in open capillaries on a Buchi Melting point apparatus and are uncorrected. IR spectra were recorded in KBr disks on a JASCO F5300 FTIR spectrophotometer.^{1}H and ^{13}C NMR spectra were recorded on a JEOL-400 MHz NMR spectrometer. The chemical shifts were reported as parts per million (ppm) with tetramethyl silane (TMS) as internal standard and coupling constants are given in Hz. Mass spectra were obtained on a Synapt UPLC-QTOF MS instrument from Waters, in positive Electron Spray Ionization (ESI) modes. Column chromatography and TLC were carried out using ACME grade 100-200 mesh silica gel and Merck precoated silica gel 60 F_{254} plates respectively

Plant Material collection

Source Collection

The leaf material area of collection of *C. Swietenia* leaves from Jaipur forest area Telangana state and collection from Gadchiroli forest area Maharashtra state, in the month of November 2019. The authenticity of the plant material was confirmed by taxonomists, Department of Botany, Kakatiya University, Hanmakonda; Geographical characteristics of the area of collection are given below **(Figure 1)**. The plant material was naturally shade dried for a period two weeks and the dried plant material was subjected to a pulverizer.

Figure 1 Area of collection of C. Swietenia leaves from Jaipur (T.S) and Gadchiroli (M.S)

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Section A:

Isolation of major secondary metabolites from the leaves of *Chloroxylon swietenia* collected from Jaipur Forest area, Telangana state (CSL-J).

Source Collection

Location	Latitude	Longitude	Altitude (Above sea level)
Jaipur Forest (Telangana)	18° 50' 47.7" N	79° 35' 12.1" E	159 meters.

Section B:

Isolation of major secondary metabolites from the leaves of *Chloroxylon swietenia* collected from Gadchiroli Forest area, Maharashtra (CSL-G)

Source Collection

Location	Latitude	Longitude	Altitude (Above sea level)
Gadchiroli Forest (Maharashtra)	20° 10' 56.66"N	80° 00' 12.06" E	217 meters

Extraction (CSL-J)

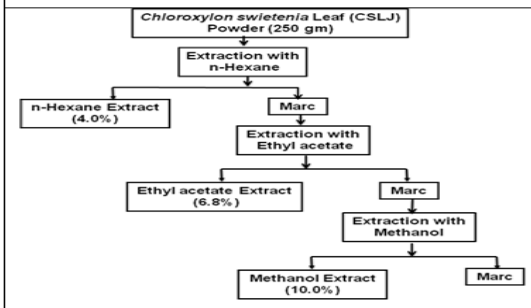
The shade dried and coarse powdered leaf of the *C. swietenia*(0.25 kg) was packed in a soxhlet extractor and extracted successively with n-hexane, ethyl acetate and methanol solvents under hot condition for 24 hr. Concentration of the three solubles under vacuum gave the respective extracts in 11 gm (4%), 17 gm (6.8%) and 25 gm (10%) respectively

Extraction (CSL-G)

The shade dried and powdered leaf of the C. Swietenia (0.5 kg) was placed in a soxhlet extractor and successively extracted with n-hexane, ethyl acetate and methanol solvents under hot condition for 24 hr. Concentration of the three solubles under vacuum gave the respective extracts in 20 gm (4%), 33 gm (6.6%) and 48 gm (9.6%), The initial TLC studies showed well resolved and different patterns to the ethyl acetate extract as compared to the n-hexane extract. Hence, ethyl acetate extract was subjected to column chromatographic separation to isolate single and pure compounds. The methanol extract could not be taken up further, as it showed unresolved TLC patterns. The three soluble portions on evaporation under reduced pressure yielded the respective extracts. Among the three extracts, the methanol extract was obtained in high yield (**Figure 2)** (10% and 9.6%), In order to identify major classes of secondary metabolites in these three extracts.

Figure 2 Employed for Extraction of *C. Swietenia leaf*

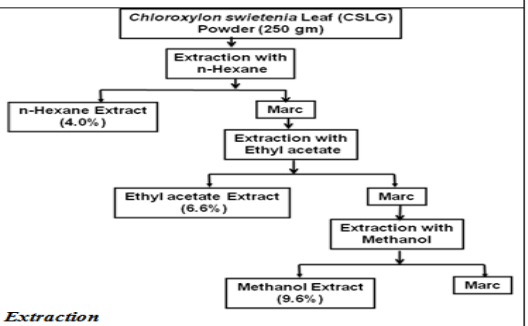
Figure 2 Protocols employed for extraction of *C.Swietenia leaf*



Extraction

The powdered *C. Swietenia* leaf material (0.25 kg) was extracted successively with different polar solvents such as n-hexane, ethyl acetate ad methanol under hot conditions by using Soxhlet extractor.

Figure 2 Protocols employed for extraction of *C. Swietenia leaf*



Extraction

The powdered *C. Swietenia* leaf material (0.5 kg) was extracted successively with different polar solvents such as n-hexane, ethyl acetate ad methanol under hot conditions using Soxhlet extractor.

Based on TLC nature, fractions F2-F6 and F2-F7 were combined and the resultant residue (15 gm) and (4.5 gm) was subjected to further column chromatographic purification over Si gel using n-hexane- ethyl acetate gradient elution to yield three single and pure compounds (**Figure 3**) CSLJ-E1, CSLJ-E2 , CSLJ-E3 and CSLG-E1, CSLG-E2, CSLG-E3..

Figure 3 Chromatographic separation of ethyl acetate extract of *C. Swietenia*

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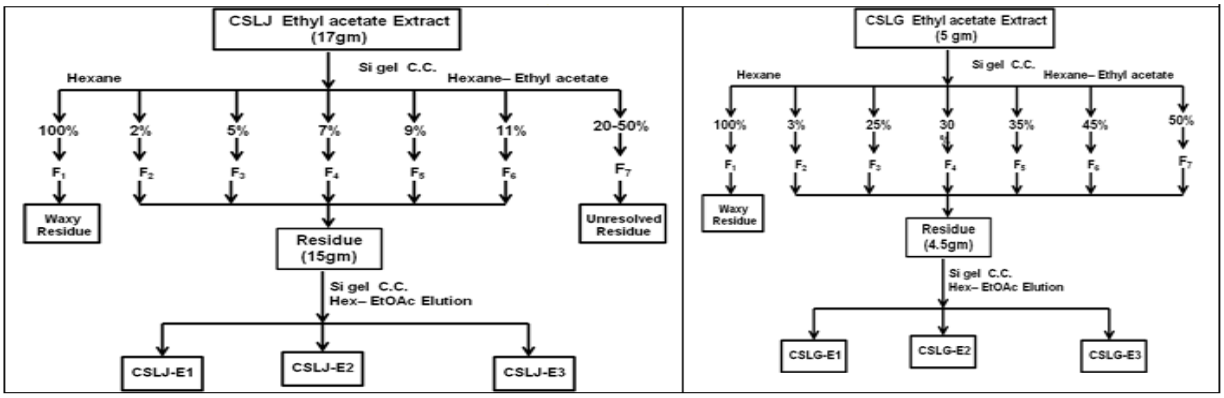

Chromatographic Separation of Ethyl acetate Extract (CSLG)

The dark green colored ethyl acetate extract (17 gm) of *C. swietenia*leaf on TLC showed some blue colored spots, but not resolved properly, as they are super imposable in n-hexane-ethyl acetate (90:10) solvent system, In order to minimize the complexicity, the extract (17 gm) was adsorbed on silica gel and fractionated over a column of silica gel (500 g, 100-200 mesh) and eluted with solvent gradient from 100% n-hexane to 50% ethyl acetate**.** Several fractions of 250 ml capacity were collected and the fractions with similar TLC nature (visualization of spots were carried out under UV light or Iodine vapours, or by spraying 5% methanolic H_2SO_4 followed by heating at 110^0 C) were combined and evaporated to yield seven major fractions (F_1-F_7)**.** Out of these fractions, F_2–F_7 showed well resolved TLC patterns. Hence, they were combined and the resultant residue (15 gm) was subjected to further column chromatographic separation using n-hexane-ethyl acetate gradient elution followed by re-crystallization with n-hexane and chloroform to yield three single and pure compounds (**CSLJ-E1 to CSLJ-E3**).

Identification of CSLJ-E1:6,8 –Diprenyl-umbelliferone

It was obtained as colour less flacks fromthe residue eluted with 5% n-hexane- ethyl acetate (95:5), 30mg (0.0017%). m.p. 230^0 C.It showed homogeneity on TLC plate (R_f: 0.5, n-hexane-ethyl acetate, 8:2).

Table 1 Identification of CSLJ-E1:6,8 –Diprenyl-umbelliferone

IR (KBr, cm^{-1})	:	1705 (C=O), 1690 and 1620.
EI Mass (m/z)	:	298 $[M^+]$
HR Mass		299.1646 $[M+H]^+$ corresponding to the Molecular formula $C_{19}H_{23}O_3$.
^{1}H NMR ($CDCL_3$,400 MHz)	:	δ 7.60 (1H, d, J=9.5 Hz), 7.08 (1H, s), 6.08 (1H, s), 6.22 (1H, d, J=9.4 Hz), 3.62 (2H, d, J=7.2 Hz), 5.29 (2H, m), 3.36 (2H, d, J=7.2 Hz), 1.87 (3H, s), 1.79 (3H, s), 1.76 (6H, s).
^{13}CNMR (DMSO+$CDCL_3$100 MHz)	:	δ 161.5 (C-2), 156.7 (C-7), 151.6 (8a),144.1 (C-4), 135.7 (C-3'), 135.0 (C-3), 125.7 (C-6), 124.3 (C-5), 120.7 (C-2'), 120.1 (C-2"), 114.2 (C-3), 112.2 (C-4a), 112.0 (C-8), 28.6 (C-1'), 25.4 (C-5"), 17.7 (C-4'), 112.0 (C-8), 28.6 (C-1'), 25.4 (C-5"), 17.7 (C-4').

Identification of CSLJ-E2: Bergaptan (4-methoxy-7H-furo [3,2-g]chromen-7-one)

It was obtained as colourless powder from the residue eluted with 7% n-hexane- ethyl acetate (93:7), 20mg (0.001%). m.p 190^0 C.It showed homogeneity on TLC plate (R_f : 0.29, n-hexane-ethyl acetate, 8:2).

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Table 2Identification of CSLJ-E2: Bergaptan (4-methoxy-7H-furo [3,2-g]chromen-7-one)

IR (KBr, cm^{-1})	:	1729 (C=O), 1625, 1582.
EI Mass (m/z) HR Mass	:	217 $[M+H]^+$ 217.04915 corresponding to the molecular formula $C_{12}H_9O_4$.
^{1}H NMR ($CDCL_3$,400 MHz)	:	δ 8.17(1H, d, J=9.9Hz), 7.60 (1H, d, J=2.3 Hz,), 7.15 (1H, s), 7.03 (1H, d, J=2.3 Hz,), 6.29 (1H, d , J=9.9Hz), 4.27 (3H, s, -OCH3).
^{13}CNMR (DMSO+$CDCL_3$100 MHz)	:	δ 161.5 (C-2), 157.8 (C-7), 152.4 (C-8a), 144.3 (C-10), 138.9 (C-4), 112.5 (C-3), 112.2 (C-5), 106 (C-4a), 104.9 (C-8), 104.7 (C-9), 93.5 (C-8), 59.66 (C-5 -OCH3).

Identification of CSLJ-E3: Isopimpinellin (4,9-dimethoxy-7H-furo[3,2-g]chromen-7-one)

It was obtained as colourless powder, from the residue eluted with n-hexane- ethyl acetate (91:9), 60 mg (0.003%). m.p 147-149^{0}C. It showed homogeneity on TLC plate. (R_f : 0.20, n-hexane:ethyl acetate, 8:2).

Table 3Identification of CSLJ-E3: Isopimpinellin (4,9-dimethoxy-7H-furo[3,2-g]chromen-7-one)

IR (KBr, cm^{-1})	:	1718.6 (C=O), 1593 (aromatic).
EI Mass (m/z) HR Mass	:	246 $[M^+]$, 247.0610 $[M+H]^+$ corresponding to the molecular formula $C_{13}H_{11}O_5$.
^{1}H NMR ($CDCL_3$,400 MHz)	:	δ 8.14 (1H, d, J=9.78 Hz), 7.63 (1H, d, J=2.32 Hz), 7.0 (1H, d, J=2.23 Hz), 6.29 (1H, d, J=9.7 Hz), 4.17 (6H, d, J=0.85 Hz,).
^{13}CNMR (DMSO+$CDCL_3$100 MHz)	:	δ 160.5 (C-2), 149.93 (C-5), 145.3 (C-7), 144.2 (C-2 '), 143.62 (C-8a), 139.3, 4 (C-4), 128.1 (C-8), 114.7 (C-6), 112.7 (C-3), 107.5 (C-4a), 105.08 (C-3), 61.6 (C-8-OCH3), 60.7 (C-5-OCH3).

Chromatographic Separation of Ethyl acetate Extract (CSLG)

The dark green colored ethyl acetate extract of *C. Swietenia leaf* on TLC showed blue colored spots, but not resolved properly, as they are super imposable in n-hexane-ethyl acetate (90:10) solvent system, In order to minimize the complexicity, the extract (5 gm) was adsorbed on silica gel and chromatographed over a column of silica gel (500 g, 100-200 mesh) and eluted with solvent gradient from 100% n-hexane to 50% ethyl acetate. Several fractions of 250 ml capacity were collected and the fractions with similar TLC nature (visualization of spots were carried out under UV light or Iodine vapours, or by spraying 5% methanolic H_2SO_4 followed by heating at 110^0 C) were combined and evaporated to yield seven major fractions (F1-F7)**.** Based on TLC nature fractions F2-F7 were combined and the resultant residue (4.5 gm) was subjected to further column chromatographic purification over Si gel using n-hexane- ethyl acetate gradient elution to yield three single and pure compounds CSLG-E1, CSLG-E2 and CSLG-E3.CSLG-E1:Racemosin (5,10-dimethoxy-8,8-dimethyl-8,9a-dihydro-pyrano[3,2-g]chromen-2(5aH)-

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one) It was obtained as colourless granules from chloroform, 80 mg (0.1 %),125-130^0 C, It showed homogeneity on TLC plate (R_f: 0.5, n-hexane-ethyl acetate, 80:20).

Table 4Chromatographic Separation of Ethyl acetate Extract (CSLG)

IR (K Br, cm^{-1})	:	1727
UV (Me OH, λ_{max}, nm)		266, 348
EI Mass (m/z)	:	311 [M+Na] suggesting the molecular formula of the compound as $C_{16}H_{16}O_5$
^{1}H NMR ($CDCL_3$, 400 MHz)	:	δ 8.15 (1H, d, H-4, J=9.9Hz), 7.63 (1H, s, H-5), 7.00 (1H, s, H-10), 6.30 (1H, d, H-3, J=9.9Hz), 4.15 (6H, s, 2 x-OCH_3), 1.25 (6H, s, 2x-CH_3).
^{13}CNMR (DMSO+$CDCl_3$,100 MHz)	:	δ 160.5 (C = O) , 150.0 (C-9a) , 145.0 (C-5), 144.8 (C-10a), 144.5 (C-3), 139.5 (C-10), 115.0 (C-6), 113.0 (C-4), 105.0 (C-4a), 61.5 (Ar-OCH_3), 60.5 (Ar-OCH_3), 29.0 (2x-methyls)

Identification of CSLG-E2:2'-Hydroxy-3'-methoxy-dihydrosuberosin [6-(2-Hydroxy-3-methoxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one]

It was obtained as a semisolid,20 mg (0.02%). It showed homogeneity on TLC plate (R_f 0.22, n-hexane:ethyl acetate , 70:30).

Table 5 Identification of CSLG-E2:2'-Hydroxy-3'-methoxy-dihydrosuberosin [6-(2-Hydroxy-3-methoxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one]

IR (KBr, cm^{-1})	:	1730
UV (MeOH, λ_{max}, nm)	:	328
EI Mass (m/z)	:	293. [M+H] suggesting the molecular formula of the compound as $C_{16}H_{20}O_5$.
^{1}H NMR (300MHz,$CDCl_3$)	:	δ 7.64 (1H, d, H-4, J=9.9Hz), 7.38 (1H, s, H-5), 6.80 (1H, s, H-8), 6.25 (1H, d, H-3, J=9.9Hz), 3.90 (3H, s, Ar-OCH_3), 3.78 (1H, m, -CHOH), 3.28 (3H, s, OCH_3), 3.00 & 2.50 (multiplets, each 1H, benzylic methylene), 1.24 (3H, s, CH_3), 1.21 (3H, s, CH_3) .
^{13}CNMR (DMSO+$CDCL_3$100 MHz)	:	δ 162.0 (C=O), 161.0 (C-7) , 154.5 (C-8a), 144.0 (C-4), 130.0 (C-5), 113.0 (C-3), 112.6 (C-6), 112.0 (C-8), 99.0 (C-3'), 75.6 (C-2'), 56.0 (C7-OCH_3), 49.5 (C3'-OCH_3), 32.0 (C-1'), 21.0 (CH_3), 19.5 (CH_3).

Identification of CSLG-E3: 2',3'-Dihydroxy-dihydrosuberosin [6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one]

It was obtained as a semisolid, 25 mg (0.025 %). It showed homogeneity on TLC plate (R_f 0.19, n-hexane-ethyl acetate, 70:30).

Table 6Identification of CSLG-E3: 2',3'-Dihydroxy-dihydrosuberosin [6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one]

IR (KBr, cm^{-1})	:	1725
UV (MeOH, λ_{max}, nm)	:	328
EI Mass (m/z)	:	279 [M+H], 301 [M+Na] suggesting the molecular formula $C_{15}H_{18}O_5$.
HR Mass		

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^{1}H NMR (300MHz,$CDCl_3$)	:	δ 7.62 (1H, d, H-4, J=9.9Hz), 7.29 (1H, s, H-5), 6.80 (1H, s, H-8), 6.23 (1H, d, H-3, J=9.9Hz), 3.90 (3H, s, 7-OCH_3), 3.75 (1H, m, H-2'), 3.01 & 2.54 (each 1H, multiplets, H-1'), 1.2-1.4 (6H, 2x-CH_3).
^{13}CNMR (DMSO+$CDCL_3$100 MHz)	:	δ 161.0 (C= O), 155.0 (C-7), 150.0 (C-8a), 143.5 (C-4), 125.5 (C-5), 110.5 (C-6, C-5a), 108.0 (C-3, C-8), 76.5 (C-3'), 62.0 (C-2'), 52.0 (C7-OCH_3), 34.0 (C-1') , 29.0 and 20.0 (C3'-CH3).

MATERIALS AND METHODS

MTT assay

To the tumor cells after respective drug treatments, 10 µl MTT (100 mg MTT/20 ml DMEM) was added and incubated for 6 h at room temperature with gentle shaking. To stop the reaction 200 µl DMSO was added and the absorbance was recorded at 590 nm in an ELISA reader. The formazan values obtained are converted to percent values, data interpreted such that decreased percentage indicate decreased survival potential. The antiproliferative potential of the tested compounds is presented as IC_{50} values and are presented in Table 3C.01.

Anti-proliferative activity pyrano-coumarin compounds (CSLG-E1-CSLG-E3)

Cell culture maintenance and treatments Human lung cancer (A-549) and neuroblastoma (IMR-32) tumor cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (50 mg/ml) in a humidified incubator chamber (37^0 C) supplied with 5% CO2. Exponentially growing tumor cells (1x104/well) in complete medium in 96-well plates were treated with 2.0, 5.0, and 10.0 µM concentrations of the three compounds **CSLG-E1**, **CSLG-E2**, and **CSLG-E3(Figure 4**). The treatments were continued for 20 h at culture conditions, and then the cells were harvested for MTT assay. Doxorubicin is used as a positive control in this assay.

Table 7 Anti-proliferative potential of furano-coumarin (CSLJ-E1-CSLJ-E3)

Compound	IC_{50} Concentration (µM)					
	SKMEL-28	**SNU-449**	**MG-63**	**HT-29**	**MDA-MB-231**	**4T1**
CSLJ-E1	**85.8 ± 1.9**	**70.37 ± 0.68**	NA	NA	**64.24 ± 1.03**	**49.9 ± 1.78**
CSLJ-E2	NA	NA	NA	NA	NA	NA
CSLJ-E3	NA	NA	NA	NA	NA	NA

NA: NOT ACTIVE

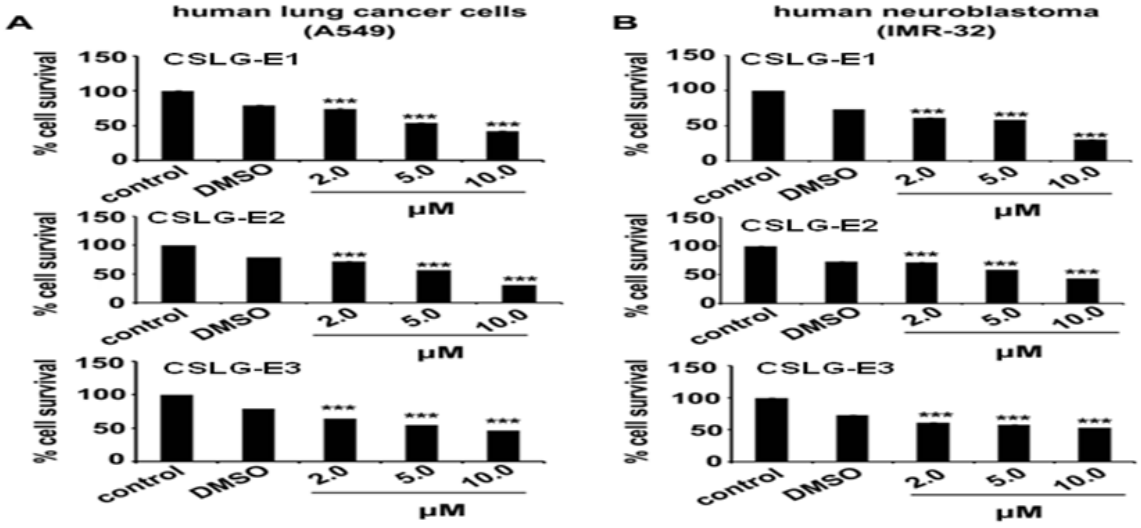

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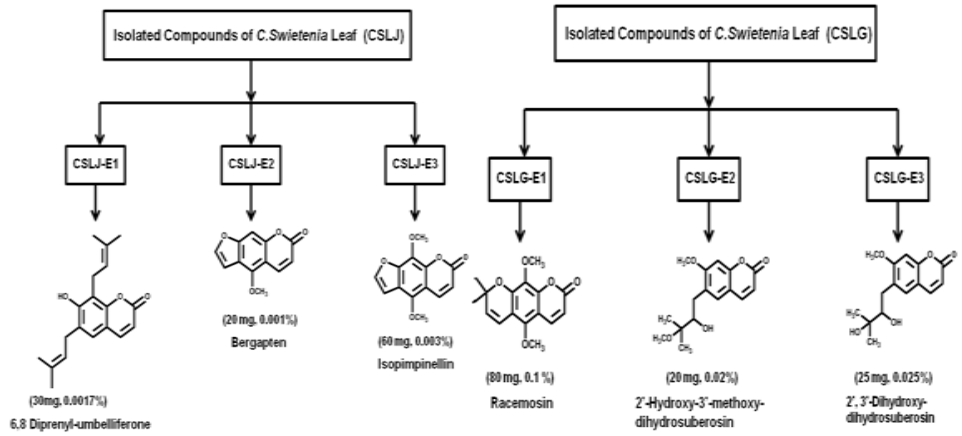
Figure 4. Effect of coumarin compounds against A549 and IMR-32 cell survival. (A) A549 and (B) IMR-32 tumor cells were treated with 2.0, 10.0, and 20.0 μM of compounds **CSLG-E1, CSLG-E2, and CSLG-E3** and the percent cell survival calculated by MTT assay. DMSO used as solvent control. X-axis represents the treatment and Y-axis represents percent cell survival after the treatment. Significance values calculated comparing DMSO controls with treatments. $p<0.001$.

From the above screening results of the compounds isolated from the leaf samples of *C. swietenia* collected from Jaipur, Telangana state (**CSLJ**) and Gadchiroli, Maharashtra state (CSLG), the Gadchiroli (CSLG) compounds such as suberosin analogues (**CSLG-E2** and **CSLG-E3**) and racemosin (**CSLG-E1**) are more active than the Jaipur (**CSLJ**) compounds. This suggests that the high altitude (217 meters) Gadchiroli sample elaborate more potent antiproliferative compounds than the relatively low altitude (159 meters) Jaipur sample. The potent molecules**(Figure 5)** from these two series are given.

Figure 5 Compounds isolated from *C. swietenia* leaves CSLJ and CSLG.



RESULTS AND DISCUSSION

From the above it is clearly seen that altitude affects the accumulation of phytoconstituents and their biological activities considerably.With this background in view, detailed and systematic chemical and biological studies were now carried out on *Chloroxylons wietenia*l eaves collected from two different altitudes. The leaves of *C. swietenia* were collected from *Jaipur* forest area,Telangana state (**CSL-J**) and from Gadchiroli, Maharashtra state (**CSL-G**). These two leaf samples were independently subjected to chemical and antiproliferative studies.

Antiproliferative activity of isolated compounds (CSLJ-E1 - CSLJ-E3 and CSLG-E1 - CSLG-E3) *Chloroxylon swietenia*

In the present investigation detailed chemical screening of the leaf samples of *Chloroxylon swietenia* collected from two different altitudes yielded two different classes of fused coumarin metabolites. The leaves of *C. swietenia*collected from Jaipur forest area, Telangana state (altitude: 159 meters) yielded exclusively the furano-coumarin metabolites (**CSLJ-E2** and **CSLJ-E3)** or its precursor (**CSLJ-E1**). Whereas the leaves collected from Gadchiroli forest area, Maharashtra state (altitude: 217 meters) yielded exclusively the pyarano-coumarin metabolite (**CSLG-E1)** or its precursors (**CSLG-E2** and **CSLG-E3**). As altitude has significant effect on the elaboration of diverse coumarin metabolites in *C. swietenia*, it was expected that it will also affect the biological activity of the metabolites isolated from its leaf samples collected from two different altitudes. Literature search reveals that significant anticancer activity was reported to both furano-coumarins [27]and pyrano-coumarins [28]. With this background, the isolated compounds of *C. swietenia* such as furano-coumarins (**CSLJ-E1–CSLJ-E3**) and pyrano-coumarins (**CSLG-E1–CSLG-E3**) were subjected to antiproliferative screening against some human cancer cell lines to identify the potent and lead compounds.

Anti-proliferative activity furano-coumarin compounds (CSLJ-E1-CSLJ-E3)

The chemical screening of the leaves of *C. swietenia* (CSLJ) collected from Jaipur, Telangana state afforded three compounds such as 6,8–diprenyl-umbelliferone (**CSLJ-E1**), bergaptan (**CSLJ-E2**) and isopimpinellin (**CSLJ-E3**). These three compounds were subjected to antiproliferative screening using MTT assay on six cancer cell lines viz.

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HT-29 (colon cancer), MDA-MB-231 (breast Cancer), MG-63(osteosarcoma), SNU-449 (hepatocellular carcinoma), SKMEL-28 (skin melanoma) and 4T1 (mouse breast carcinoma). Close analysis of the above data reveals that compound **CSLJ-E1** (6,8–diprenyl-umbelliferone) showed moderate activity against four cancer cell lines such as SKMEL-28 (skin melanoma), SNU-449 (hepatocellular carcinoma), MDA-MB-231 (breast Cancer) and 4T1 (mouse breast carcinoma). Surprisingly, the other two compounds, **CSLJ-E2** and **CSLJ-E3** did not show any activity. Interestingly, **CSLJ-E1** (6,8–diprenyl-umbelliferone) showed highest activity against 4T1 (mouse breast carcinoma) with an IC_{50} of 49.9 ± 1.78 followed by MDA-MB-231 (breast Cancer), SNU-449 (hepatocellular carcinoma) and SKMEL-28 (skin melanoma) with IC_{50} values 64.24 ± 1.03, 70.37 ± 0.68 and 85.8 ± 1.9 respectively. The screening data also reveals that the precursor ortho-hydroxydiprenyl compound (**CSLJ-E1**) exhibited moderate activity, whereas the cyclised fused fuaranocoumarins did not show any activity.

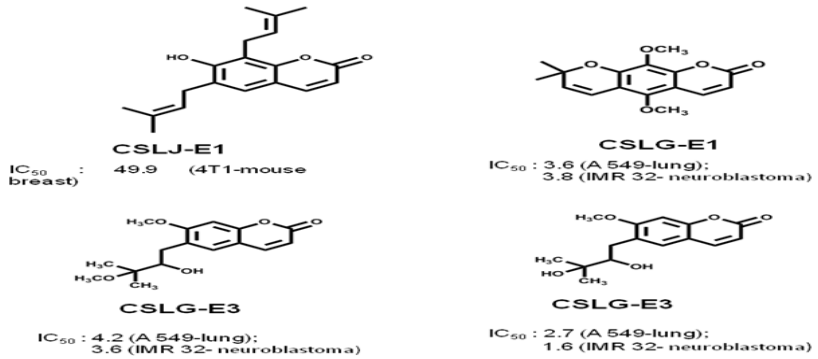
Anti-proliferative activity pyrano-coumarin compounds (CSLG-E1-CSLG-E3)

The chemical screening of the leaves of *C. swietenia* (**CSLJ**) collected from Gadchiroli Forest; Maharashtra state afforded three compounds such as racemosin (**CSLG-E1**), 2'-Hydroxy-3'-methoxy-dihydrosuberosin (**CSLG-E2**) and 2', 3'-Dihydroxy-dihydrosuberosin (**CSLG-E3**). These three compounds were subjected to antiproliferative screening using MTT assay on two human cell lines viz. A-549 (lung cancer) and IMR-32 (neuroblastoma cell line). The details of the assay are presented in Table 8 and Figure 6 was identified as potent compounds with interesting functional groups

Table 8 Anti-proliferative potential of pyrano-coumarin (CSLG-E1-CSLG-E3)

Cell line	CSLG-E1	CSLG-E2	CSLG-E3	Doxorubicin
A-549	**3.6**	**4.2**	**2.7**	**2.2**
IMR-32	**3.8**	**3.6**	**1.6**	**1.9**

Figure 6 Compounds CSLJ-E1 and CSLJ-E3, CSLG-E1 and CSLG-E3 with interesting functional groups have potentiality as anticancer therapeutic agents.



MOLECULAR DOCKING

Docking results are becoming more precise these days. Computational chemistry is becoming increasingly difficult for medicinal chemists, and it produces rapid findings. In my research, I was used 5ZSY (LUNG CANCER) and 4TUL (NEUROBLASTOMA) proteins from the Protein Data Bank (pdb) RCSB website (29) for my study. When I compared my isolated products from the aforesaid protein to DOXYRUBICIN,(Table 9) it showed the same findings as the reference. Lung cancer showed identical results when compared to doxyrubicin. I utilised the 0.8 version of Pyrx software, which has academic licences. (30)

Table 9 Showing the proteins with standard reference

Compound	5ZSY	4TUL

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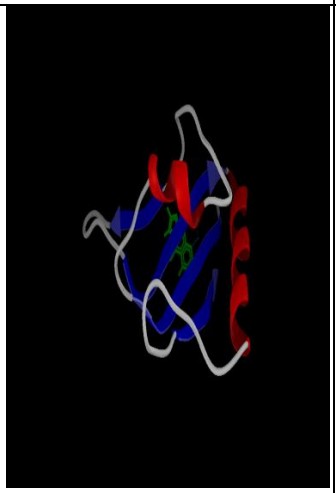
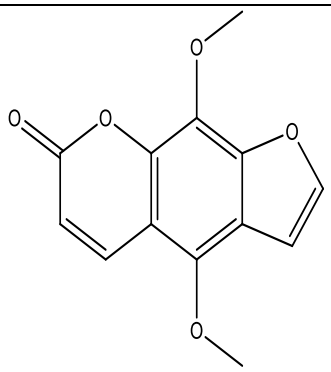
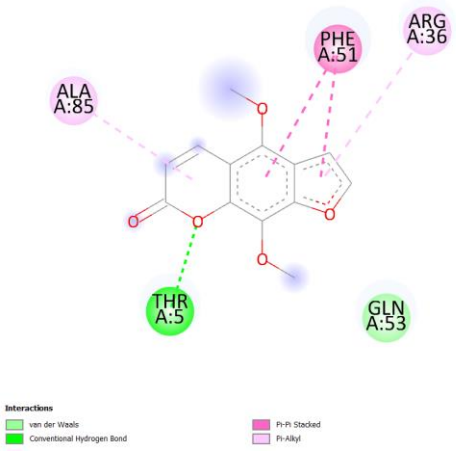
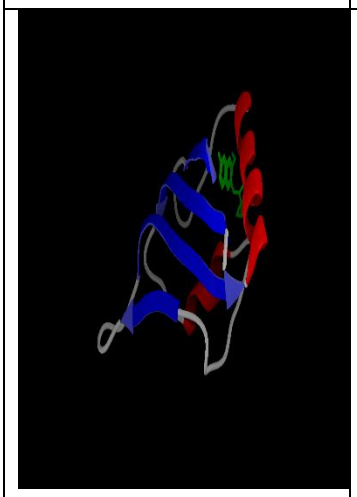
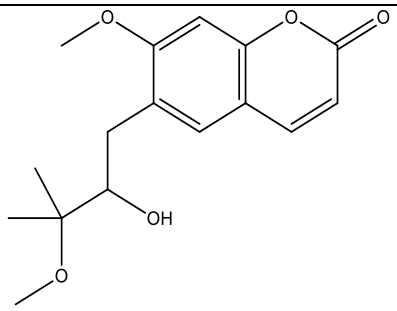
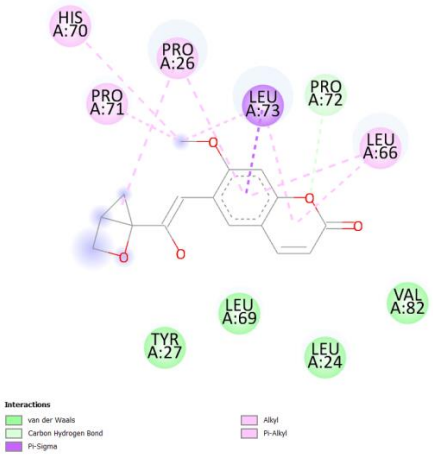
CSLG-E1	-5.1	-6.4
CSLG-E2	-6.6	-6.9
CSLG-E3	-5.9	-6.9
DOXORUBICIN	-6.6	-8.3

Table 10 Showing 2d structure of protein binding and Active site of Binding from isolated structures.

2D STRUCTURE OF PROTEIN BINDING	ISOLATED STRUCTURE	ACTIVE SITE OF PROTEIN BINDING
	 4,9-dimethoxy-7H-furo[3,2-g]chromen-7-one CSLG-E1	
	 6-(2-hydroxy-3-methoxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one CSLG-E2	

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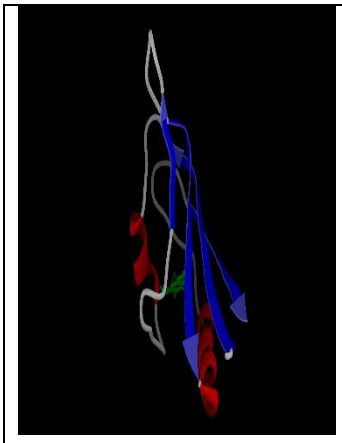
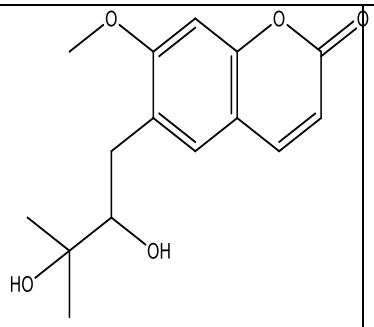
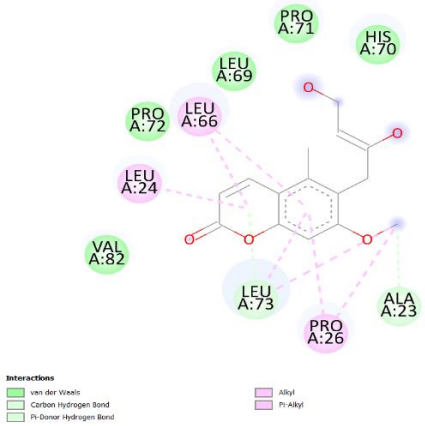
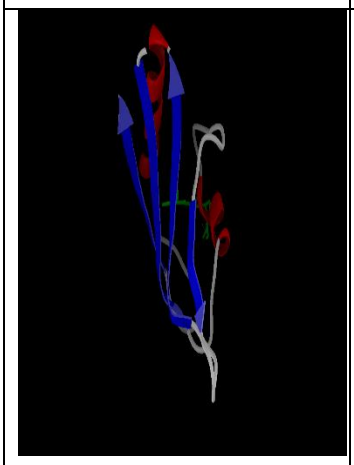
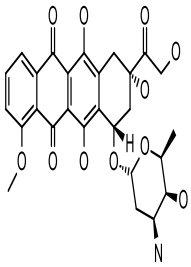
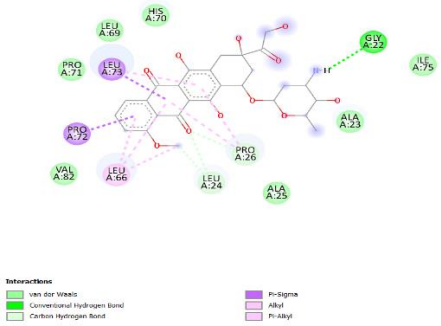
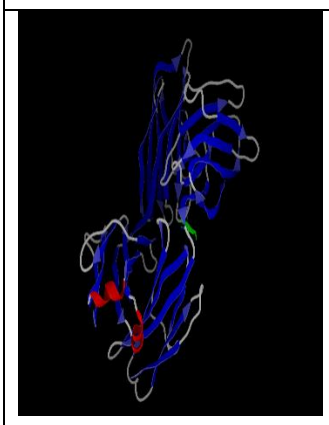
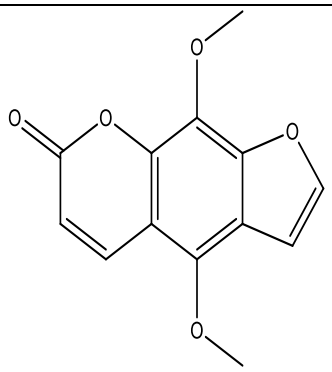
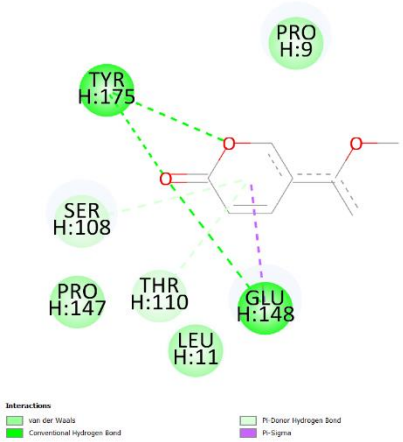
	 6-(2,3-dihydroxy-3-methylbutyl)-7-methoxy-2H-chro CSLG-E3	
	 (8 *S*,10 *S*)-10-((2 *R*,4 *S*,5 *S*,6 *S*)-4-amino-tetrahydro-5-hydroxy-6-methyl-2 pyran-2-yloxy)-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(2-hydroxyacetyl)- DOXYRUBICIN	
	 4,9-dimethoxy-7H-furo[3,2-g]chromen-7-one CSLG-E1	

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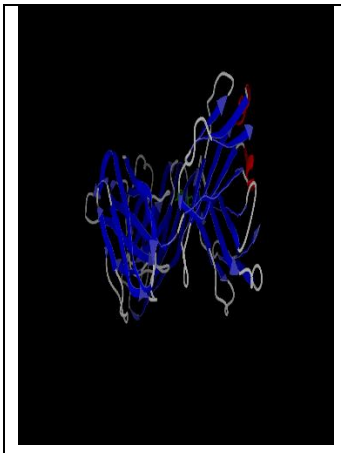
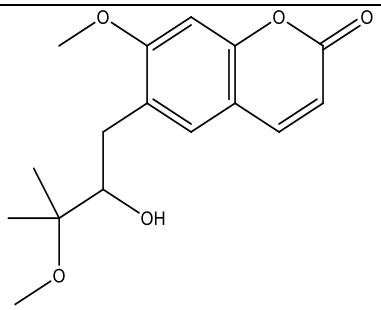
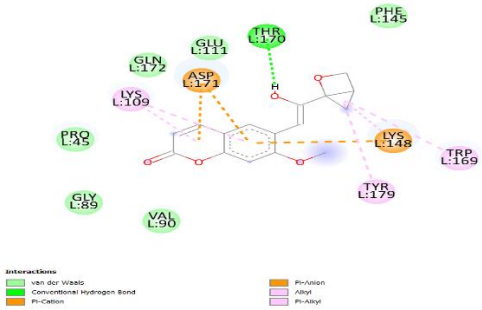
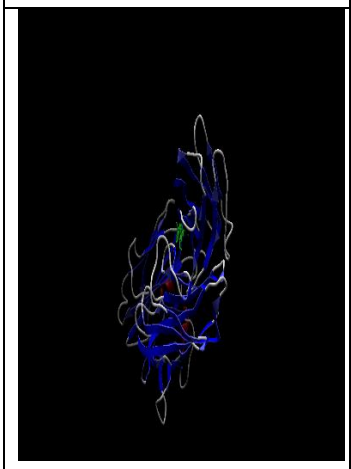
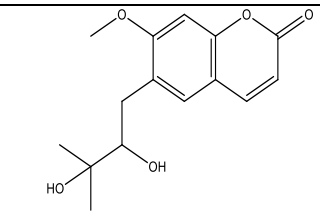
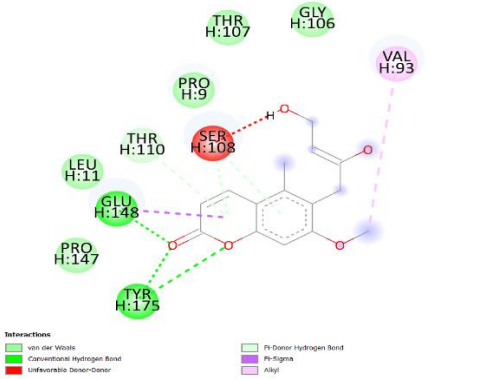
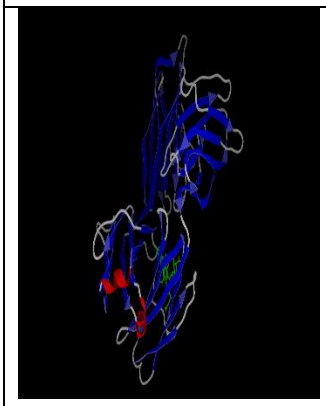
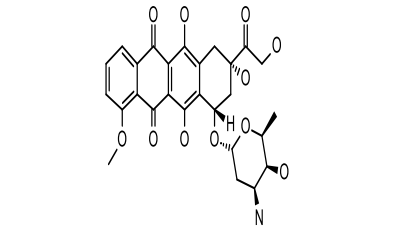
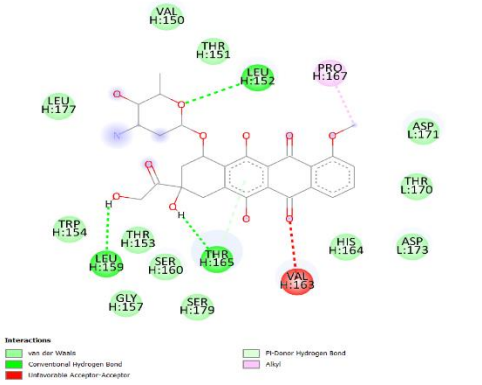
	 6-(2-hydroxy-3-methoxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one CSLG-E2	
	 6-(2,3-dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one CSLG-E3	
	 (8 S,10 S)-10-((2 *R*,4 S,5 S,6 S)-4-amino-tetrahydro-5-hydroxy-6-methyl-2 pyran-2-yloxy)-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(2-hydroxyacetyl)- DOXYRUBICIN	

CONCLUSION

The abiotic parameter, altitude is reported to effect the elaboration of secondary metabolite in natural plant species. In order to validate this concept, the detailed chemical and biological studies have now been carried out on the leaves of *Chloroxylonswietenia*, one of the potent Indian folklore medicinal plant. The leaf samples of *Chloroxylonswietenia* were collected from two different altitudes. The leaves of *C. swieteniac*ollected from Jaipur forest area (altitude: 159 meters), Telangana state yielded exclusively the furano-coumarin metabolites (CSLJ-E2 and CSLJ-E3) or its precursor (CSLJ-E1). Whereas, the leaves collected from Gadchiroli forest area (altitude: 217 meters), Maharashtra state yielded exclusively the pyarano-coumarin metabolite (CSLG-E1) or its precursors

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(CSLG-E2 and CSLG-E3). As significant anticancer activity was reported in literature to both furano-coumarins and pyrano-coumarins, the isolated compounds of *C. swietenia* were now subjected to antiproliferative screening against some human cancer cell lines to identify potent lead compounds and docking studies Invivo studies only two cell lines are active. i.e I did only two protein docked 5ZSY (LUNG CANCER) (-6.6) 4TUL (NEUROBLASTOMA) lung cancer shows similar results as reference doxyrubicin (-6-6) The Gadchiroli (CSLG) compounds such as suberosin analogues (CSLG-E2 and CSLG-E3) and racemosin (CSLG-E1) are more active than the Jaipur (CSLJ) compounds. This suggests that the high altitude (217 meters) Gadchiroli sample elaborate more potent antiproliferative compounds than the relatively low altitude (159 meters) Jaipur sample. The identified potent compounds with interesting functional groups have potentiality to develop further as anticancer therapeutic agents.

Statistical analysis of data

Data in the bar diagrams are reported as mean±SD. The control groups are compared with treated ones and the significance values were calculated by paired student's t-test. Data represented are from minimum three independent sets of experiments in each group. The *p*values $p<0.05$; ,$p<0.01$, and, $p<0.00$

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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