Unravelling the therapeutic potential of *Lantana camara* in skin cancer


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Abstract

Natural products and their metabolites have a historical significance as they serve as a starting point for drug discovery and development process. *Lantana camara*, a notorious invasive weed has been used in folk medicine for managing various health concerns. Pentacyclic triterpenoids (Lantadenes), have attracted significant interest due to their anticancer potential. The present study include *in vivo* anticancer investigations of 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid using two-stage carcinoma model. This compound showed notable anticancer potential, evidenced by a substantial reduction in tumour burden and volume along with attenuation in proliferation, thickness, and corrugation of epidermis in cancer model. These findings indicate Lantadene structural modification may hold substantial potential against skin malignancies.

Keywords: Lantadenes, Weed, Natural products, Anticancer

1. Introduction

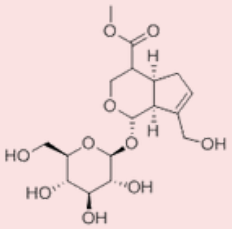
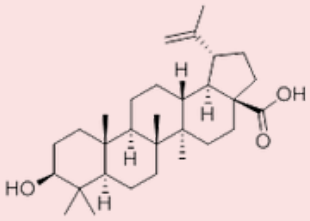
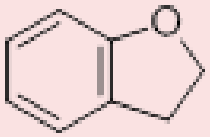
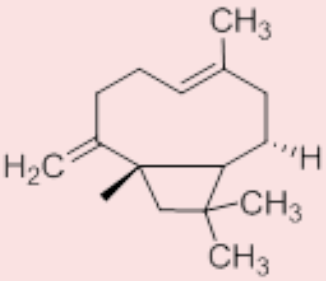
Mother nature serves as an endless reservoir for new chemotypes and pharmacophores. Natural metabolites and their derivatives have historically made a major contribution in pharmacotherapy, specifically in infectious and cancer diseases (1,2). Natural product (NP) databases exemplifies structural and qualitative activity information for approximately 4,70,000 NPs, while around 5,000 NPs are reported with experimental values only, highlighting a significant untapped profile of natural products (3,4). The use of natural products for medical purposes is dated back dawn to human existence. The utilization of plants as therapeutic agents is even evidenced by the murals found in the Lascaux caves, dated back to 20,000 B.C. (5).

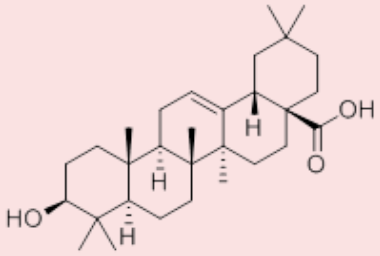
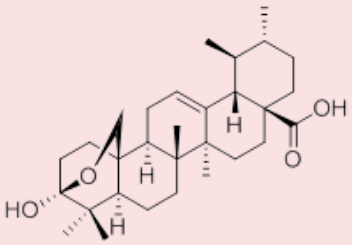
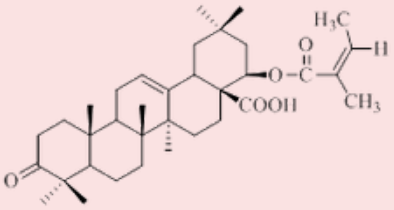
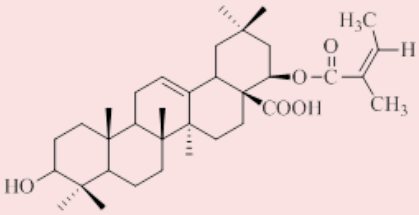
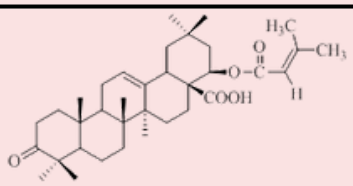
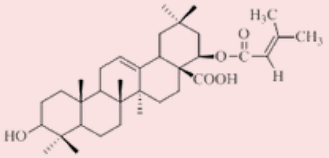
Plants provide an extensive chemical space filled with therapeutic potential, and emerging as a topic of global significance in drug discovery process. Plant-derived metabolites have significantly contributed in the treatment of numerous ailments (6). The potential benefits of these secondary metabolites are owed to their remarkable stereochemistry, structural diversity and extensive range of pharmacophore enhances receptor binding selectivity, offering these natural scaffolds as a valuable source for novel pharmaceuticals (7). As far as plants are concerned, the significance of weeds in the pharmacopoeia has been under-valued despite of strong evidence that weeds are being used as medicines among indigenous people (8). Numerous investigations have indicated that weeds are rather rich in secondary metabolites, hence they may have great potential for therapeutic development (9).

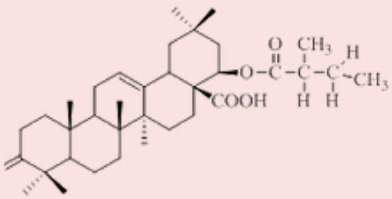
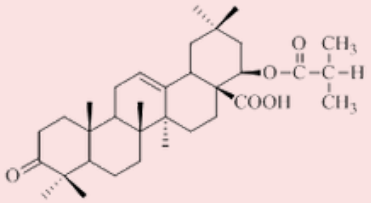
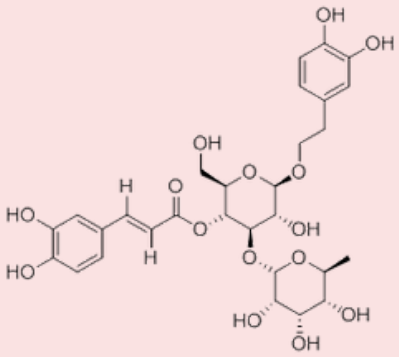
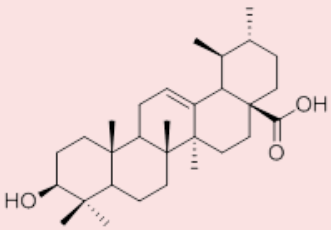
Lantana camara, a member of the Verbenaceae family, is a notorious weed that has attracted significant attention among the scientists since the last two decades (10). *Lantana camara*, also known as red sage, wild sage or lantana, is considered as one of the most noxious weeds globally (11). Linnaeus created the genus Lantana in 1753, and Chamisso subsequently defined the subgenus Camara in 1832 (12). Lantana is indigenous to tropical & subtropical America being distributed across 60 nations and reported with approximately 650 varieties (13).

During early 1690s, Lantana began to spread across the entire world through trade and commerce. According to Kohli et al., first time Lantana was brought to India during 1807 in Kolkata, while introducing variety of plants to the botanical gardens (14). Further, the modern medical system has started to acknowledge the beneficial effects of Lantana (15). Lantana has been extensively reported for its potential as anti-cancer, anti-ulcer, anti-malarial, anti-microbial, nematicidal, anti-hyperglycaemic, wound healing potentials, and anti-hypertensive etc. shown in Table 1.

Table 1. Phytochemicals from *Lantana camara* and their pharmacological activity.

Plant parts	Phytochemical	Pharmacological activity	Ref
Roots	 Geniposide	Hypolipidemic activity	(15)
Aerial parts, stem	 Betulinic acid	Anti-leishmanial, cytotoxic, nematicidal activity	(16)
Leaves	 Coumaran	Insecticidal, Anti-acetylcholinesterase activity	(17)
Leaves	 *E*-Caryophyllene	Bactericidal, anti-fungal, antimicrobial, anticancer activity	(18)

Aerial parts, stem, roots	 Oleanolic acid	Antiurolithiatic antimicrobial, hepatoprotective,anti-inflammatory, antihyperlipidemic, antifertility, antimicrobial,antiulcer, nematicidal activity	(19-21)
Leaves	 Lantic acid	Antibacterial activity	(22)
Stem, leaves, roots	 Lantadene A	Anti-leshmanial, Nematicidal, anti-microbial, antitubercular, anti-cancer activity	(23-25)
Leaves	 Reduced Lantadene A	Antiviral, cytotoxic activity	(12,26)
Stem, leaves	 Lantadene B	Antiviral, hepatotoxicity, nematicidal antimicrobial, allelopathy, antitumor activity	(27,28)
Leaves	 Reduced Lantadene B	Anticancer potential	(12,26)

Stem, leaves,	 Lantadene C	Antiviral activity	(28)
Stem	 Lantadene D	Anticancer activity	(23)
Stem, leaves	 Verbascoside	Vasodilator, antifungal, Cardiotonic, protein kinase C inhibitor,antihypertensive, anti-inflammatory, anticancer	(29)
Leaves	 Urs-12-en-3β-ol-28-oic acid 3β-D-glucopyranosyl-4-octadecanoate	Anti-diabetic activity	(30)

Pentacyclic terpenoids, specifically Lantadene A and B (Figure1), have garnered significant interest due to their promising cytotoxic properties. Over the past decade, various structural modifications were implemented at C-2, C-3, C-17, C-22 positions in rings A and D, demonstrating anti-inflammatory and anti-tumor properties via downregulation of B-cell lymphoma-2, Nuclear factor kappa B, c-Jun, and the inhibition of Akt protein (27,28). Research from Sharma et al indicated by keeping ester side chain at C-22 and modifying structural variations at C-3 position of Lantadene analogues, revealed significant anti-cancer potential. The remarkable anticancer impact of these compounds is attributed to the predicted binding association of the C-22 ester linkage with the binding domain of receptor that modulate the physiological response (28,31).

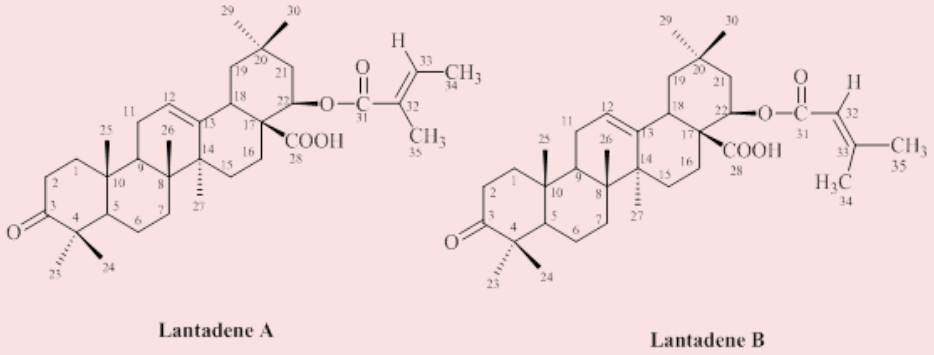


Figure 1. Lantadenes

Exploiting *in silico* studies, we have disclosed 2 & 3 Dimensional quantitative structure-activity relationship investigations, highlighting pharmacophoric features that govern anti-cancer potential (31). Subsequently collective *in vitro* & *in silico* analysis of our recently published research article on synthesis of C-3 ester analogues of Lantadenes have identified 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid, as a lead molecule (32). Thus the current study, attempted to investigate anti-cancer efficacy of 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid in model of skin carcinogenesis.

2. Experimental

2.1. *In vivo* studies

The anti-cancer activity of 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid was evaluated in Swiss albino mice (LACCA) using a skin carcinogenesis (two-stage) model. The Animal Ethics Committee, Panjab University, Chandigarh (PU/45/99/CPCSEA/IAEC/2018/120) evaluated & approved all procedures for *in vivo* studies and CPCSEA standards were followed for animal care, handling, and experimentation. Depilatory lotion was used on the dorsal skin of mice to eliminate hair.

Mice in control (Group I; n=5) served as control and received only acetone (100µL) as vehicle treatment biweekly for 25 weeks. Group II (n=14) animals received 100nmol 7,12-Dimethylbenz[a]anthracene (DMBA) biweekly for 2 weeks, subsequently followed by 1.7nmol 12-O-tetradecanoylphorbol-13-acetate (TPA) administration biweekly for the next 23 weeks. Group III (DMBA/TPA + compound; n=14)animals received 50mg/Kg body weight of mice with 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid from 4 week onwards and continued till 25thweek. After the termination of protocol tumor incidence %, mean tumor volume and burden were analysed. Further, Histoarchitechtural investigation were performed by fixing of skin & tumors samples in Zenker and staining with hematoxylin and eosin dye.

3.Result and discussion

3.1. *In vivo* studies

The topical treatment of DMBA/TPA over 25 weeks led to tumour development, with the exception of control group I. All animals were monitored for lesions, papillomas, and tumours during the treatment. Figure 2 shows various morphological transformations in skin cancer during the protocol. The first appearance of papillomas, average tumor count, and animal mortality were recorded weekly for the period of 25 weeks. Additionally, tumour burden and volume were evaluated at end of protocol. In group II animals, papillomas appeared in the sixth week and progressed from 26 to 100% by eleventh week. On the other hand, group-III animals emerged with 13% at week six and escalated to 53% by the end of the protocol. Administration of compound attenuated tumour incidence by 47% in group III animals compared to group II. Further, results from Table 2 demonstrated the mean tumor volume ranged from 30-716 mm^3 & 17-67 mm^3 in the group-II group-III animals respectively. Treatment with compound indicated a substantial reduction in tumor volume with a maximal volume of 67 mm^3 compared to group II animal.

In the similar direction mean tumor burden was calculated and varied from 67-1431 mm^3 & 15-134 mm^3 for group-II & group-III, respectively. Supporting tumor volume parameter, treatment group significantly decreased tumour burden, with a maximum of 134 mm^3 compared to 1431 mm^3 in group II (Table 2).

Table 2. Impact of 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid on DMBA/TPA induced skin tumors

Parameters	Tumor incidence (%)	Tumor burden (mm^3)	Tumor Volume (mm^3)
Group I	NA	NA	NA
Group II	100%	Range: 67-1431 (766 ± 606)	Range: 30-716 (301±274)
Group III	53%	Range:15-134 (48±45 [b])	Range: 17-67 (31 ± 20 [a])

Data is reported as Mean ± SD (n=14) using Student's t-test. [a]p≤0.01& [b]p≤0.05 significant with respect to group II.

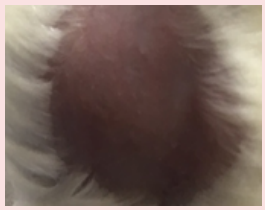
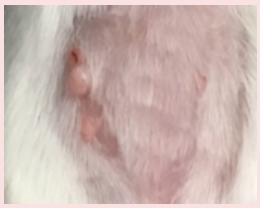
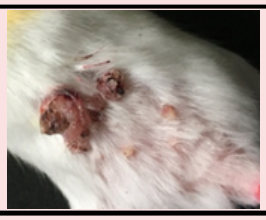
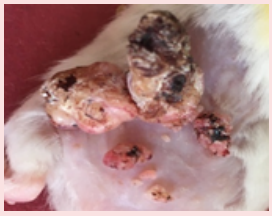
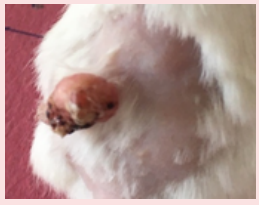
	(Group-II)	(Group-III)
Normal Skin		
1st incidence of lesion at 6th week		
Developing phase during 10th week		
Tumor in the termination stage at 25th week		

Figure 2. Morphological transformations in skin cancer using skin carcinogenesis model

3.2. Histological investigation of normal skin and skin tumours

Histoarchitechtural study was performed on skin samples from group-I and tumor samples from group-II & III tumors. Harvested samples were regularly processed, fixed followed by embedding in Zenker and paraffin respectively. Histopathological examinations were performed under a light microscope using hemoatoxylin and eosin-stained sections.

The administration of DMBA followed by TPA biweekly on bare skin led to tumors development in both group II & group III mice, while no tumors were noticed in the control group. Uniformly arranged intact epidermis, underlying layers & subcutaneous tissues were found in group I samples. Tumours from group II & group III were darkly pigmented with hyperchromatic nuclei, indicating a nucleus-to-cytoplasm ratio favouring the nucleus. Well-defined epidermal carcinoma was identified in group II, characterised by thickened keratinized layer on the epidermis (hyperkeratosis) and corrugated epidermis resulting from hyperproliferation from normal to hyperplastic states. These tumors exhibited a profusion of keratin pearls and substantial infiltration of malignant cells into the underlying dermis, demonstrating a key characteristic of epidermal keratinocyte tumours, specifically squamous cell carcinoma (Figure 3). Histopathological investigation from group III samples verified the existence of squamous cell cancer. Significantly proliferating, corrugated and thickened epidermis was noted. However, epidermis exhibited reduced thickening and markedly decreased keratinisation. Furthermore, vacuoles resulting from degenerative alterations were observed in the tumors retrieved from group III mice. The extent and amount of hyperchromasia was significantly declined in comparison to group II.

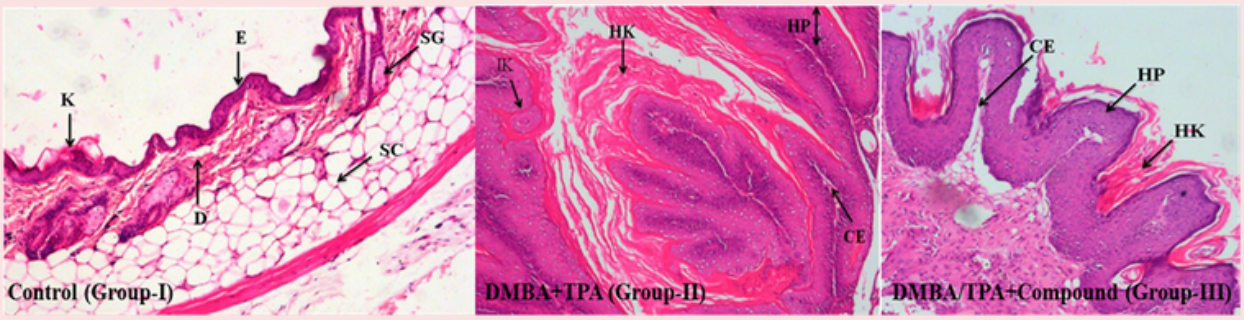


Figure 3. Histoarchitecture samples from group-I, group-II and group-III at 10X magnification: epidermis (E), dermis (D), keratinocytes (K), subcutaneous tissue (SC), Island of epidermal cells encapsulated with keratin (IK), hyperkeratinisation (HK), corrugated epidermis (CE), sebaceous glands (SG), hyperproliferative epidermis (HP).

In vivo investigations indicated that administration of 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid to tumor-bearing mice considerably decreased tumour incidence, indicating chemo preventive action of compound. Further, studies also pointed out that presence of empty spaces in tumours that lead to significant decrease in tumour volume and tumour burden after the treatment may be associated with degrative action of compound.

4. Conclusion

Despite significant advancement, anti-cancer tailored small molecules still suffers from substantial adverse effects. Simultaneously, growing interest in indigenous traditional herbal remedies is emerging as an alternative for the treatment and prevention of various illnesses. Weed Lantana has garnered attention from decades due to its anticancer potential. Observation of this study indicated promising anticancer potential with significant reductions tumour volume, and burden. Further, histopathological investigations revealed attenuation in proliferation, thickness, and corrugation of epidermis indicating 3β-(4-Methoxybenzoyloxy)-22β-senecioyloxy-olean-12-en-28-oic acid as potential candidate against skin cancer. However, additional research is necessary to exhaustively explore the underlying mechanisms and pharmacokinetics studies.

Acknowledgments

Authors are grateful to the University Institute of Pharmaceutical Sciences, Panjab University for providing the infrastructure and highly equipped lab facilities.

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