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# Characterization of a Bioactive Triterpenoid from the Toothache Plant, *Acmella oleraceae*, and Identification as an Anthelmintic Compound

**Running Title:** Anthelmintic triterpenoid from *Acmella oleraceae*

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## ABSTRACT
Helminthiasis is a leading parasitic infection in animals and humans that causes a persistent global crisis, aggravated by pervasive drug resistance in the most harmful species. *Acmella oleracea* (L.) R.K. Jansen is a valuable traditional medicinal plant for treating helminthiasis. The study aimed to identify the chemical compound responsible for the anthelmintic property. *A. oleracea* extract was prepared with hexane and fractionated in column chromatography by a series of elution with hexane and ethyl acetate. The isolated compound was characterized with elemental analyzer, mass spectrometry, Fourier-transform nuclear magnetic resonance spectroscopy, Fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. The anthelmintic activity was tested against an intestinal tapeworm of chicken and the effects were studied using scanning electron microscopy. Chemical analyses indicated that the compound was a pentacyclic triterpene showing the structure of lup-20(29)-en-3-ol. This is the first identification of the compound from *A. oleracea*. The anthelmintic activity was compared with that of albendazole and showed a potent efficacy against a poultry tapeworm. Scanning electron microscopy revealed signature anthelmintic damages throughout the body surface of the tapeworm including destruction of the tegument, degeneration of the suckers and removal of the spines. Our findings show that the isolated compound is the anthelmintic principle of *A. oleracea* as used in the Mizo traditional medicine. Further studies on the exact mechanism of action, cellular effects and pharmacological properties are required for anthelmintic development.
**Keywords:** Chromatography, Mass spectrometry, Scanning electron microscopy, Tapeworm

## INTRODUCTION
Helminthiasis is a collective disease among the neglected tropical diseases that is due to infections with diverse groups of helminth parasites, broadly classified under two major phyla in animal kingdom, Platyhelminthes (comprising tapeworms and flukes) and Nematoda (consisting of all roundworms). With an estimated 1.5 billion people affected at any moment, it is the most prevalent infection causing debilitating health concerns in humans and veterinary animals worldwide [1]. Despite extensive global campaigns and deworming programs, the disease remains an insidious crisis as the parasites acquired resistance to the available anthelmintic drugs in animals, compounded by reports of reduced drug efficacy in humans [2,3]. Helminths have evolved various drug evasion mechanisms such as increased elimination of drug molecules, faster drug metabolism, inhibition of or weakened drug receptors, gene repression of the drug receptors, and reduced affinity for drug uptake [4]. The gravest concern in animals is the subtle spread of multi-drug resistance in the most impactful parasites [5]. Considering the complexity of the life cycle stages in different helminths [6], it does not appear to be a simple feat of drug development to produce a totally effective medication and the need for one, or at least an improved therapeutic, is a fundamental quest in the management of helminthiasis [7].
The genus *Acmella* consists of several species of small annual herbs belonging to the family Asteraceae native to South America that eventually became widely distributed in Asia. The plants are popularly called toothache plants for their medicinal use in dental care. In different Asian traditional systems, they are also used as culinary plants and for various therapeutic purposes [8]. The most well-known species, *Acmella oleracea* (L.) R.K. Jansen, is one of the most multifaceted medicinal plants that is traditionally employed in the treatments of blood diseases, cancer, diuresis, febrile illness, immune diseases, indigestion, liver infection, and peptic ulcer [9,10]. In some cultures, it also known for its properties such as analgesic, antimalarial, antimicrobial, antivenom to snake bites and insecticide against mosquitos [9,11]. Its analgesic and soothing menthol-like flavor is used in different conditions of dental and mouth problems and is commercially produced for use in dental health care – the basis of the popular name, toothache plant [12]. It also exhibits a botulinum neurotoxin-like effect by inducing muscle relaxation, the property of which is commercialized as a safer and cheaper alternative to botox in cosmetic treatments, and aptly earned the name herbal botox [13].
The Mizo people live in Mizoram, Manipur and Tripura, which are the remotest states in northeast India, and in Chin State of Myanmar. Their habitation falls under the Indo-Burma biodiversity hotspot that serves as the corridor between mainland India, the Himalayan biodiversity hotspot and Asia for species migration [14]. In the Mizo traditional system, the whole plant of *A. oleracea* is a popular vegetable that is consumed raw or boiled. It is a treasured medicinal plant for curing gastrointestinal infections, dental diseases, dysentery, migraine, oral infection, rheumatoid arthritis and disfluency in children. It has a strong pungent odor that is applied in farms and households

as a deterrent to insects [15,16]. The most distinctive usage is as an indigenous deworming agent as it is known to be effective against both tapeworm and roundworm infections of the bowel [17]. In the light of this medicinal property, we have isolated some compounds, one of which was a pentacyclic triterpene. We performed anthelmintic assay to determine whether the compound is involved in the bioactivity of the plant against helminth parasites.

## MATERIAL AND METHODS

### Chemicals and Drugs

All chemicals in the study were of standard analytical grades manufactured by HiMedia Limited, Mumbai, India. Chromatographic grades of acetonitrile, chloroform-d, and tetramethylsilane were obtained from Merck (Sigma-Aldrich), Mumbai, India. Standard anthelmintic, Zenlee® (albendazole) was purchased from UNI-PEX, New Delhi, India.

### Ethical Approval

Parasitic tapeworms recovered from local chickens were used for anthelmintic study according to the approval of the Institutional Animal Ethics Committee of Pachhunga University College (no. PUC-IAEC-2016-Z2).

### Plant Specimen and Extraction

The leaves and flowers of *A. oleracea* were obtained from an agricultural farm in Ngopa at Saitual district, Mizoram, India (location 23.8861°N and 93.2119°E). Herbarium specimens were prepared and validated for taxonomic identity at the Botanical Survey of India, Eastern Regional Office, Meghalaya, India (no BSI/ERC/Tech/Ident/2017/312*)* and preserved in the botanical herbaria collection of Pachhunga University College, bearing a catalogue number PUC-A-17-1. The plant parts for extraction were dried in ambient environment at 22 ± 2°C in a humidity-controlled chamber for four weeks. Extraction was performed in a Soxhlet apparatus of 5-L capacity using hexane. A slurry of crude extract was collected and concentrated in Buchi Rotavapor® R-215 (Flawil, Switzerland) under reduced pressure.

### Compound Isolation

*A. oleracea* extract was mixed with silica gel of 60-120 mesh size and loaded into a pre-fixed glass column of 60 cm long and 4 cm bore size packed with activated silica gel of 200-430 mesh size. The column was eluted first with hexane followed by a mixture of hexane and ethyl acetate, specifically 0.1, 0.2, 0.3, 0.4 and 0.5% ethyl acetate. A total of 60 fractions were collected. From each fraction, 250 mL was taken out and analyzed using thin-layer chromatography (TLC) to detect the compounds. The fractions obtained from elution with 0.5% ethyl acetate in hexane indicated the major compound. The final compound was visualized at 254 and 366 nm in ultraviolet (UV) and iodine chambers.

### Thin-layer Chromatography

All fractions of the plant extracts were examined with TLC to identify the compound. The compound was spotted on pre-coated Sigma-Aldrich® (Darmstadt, Germany) TLC Plates (20 × 20 cm, pore size 60 Å, 200 µm thick). The plates were developed using hexane: ethyl acetate mixture at various ratios and visualized in a UV cabinet at 254 and 366 nm.

### Elemental Analysis

Elements such as carbon, hydrogen, nitrogen, sulphur and oxygen were analyzed for the isolated compound in a CHNS-O elemental analyzer, EuroVector EA3000 (Pavia, Italy). Samples were treated with 1% hydrochloric acid and repeatedly washed with Milli-Q® water (Merck, Darmstadt, Germany). Helium was used as a carrier gas for the solution. The dried samples were subjected to controlled combustion in TurboFlash™. Data were generated from Callidus® software.

### Mass Spectrometry

Molecular mass of the compound was analyzed using atmospheric-pressure chemical ionization (APCI) associated with electrospray ionization (ESI) and high-resolution mass spectrometry (HRMS) in Agilent 6520 accurate-mass Q-TOF LC/MS system coupled to Agilent 1200 high-performance liquid chromatography (Santa Clara, California, USA). Acetonitrile was used as a solvent. Injection volume was made at 1 µL, at a flow rate of 150 µL/min. The temperature of the ion source interface was set at 340°C. The drying gas, nitrogen, was run at a flow rate of 4 L/min, capillary voltage was set at −1952 V, scan rate maintained at 3.225 scan/sec at 4 GHz mode covering a scan range of 100–700 *m*/*z*. Spectral analysis was done in Agilent MassHunter Workstation software.

### Fourier-transform Nuclear Magnetic Resonance Spectroscopy (FT-NMR)

One-dimensional $^{1}H$ and $^{13}C$ NMR spectra were generated from Bruker Avance II, 400MHz FT-NMR (Billerica, Massachusetts, USA). 10 mg of the compound for $^{1}H$ and 50 mg for $^{13}C$ were dissolved in deuterated chloroform ($CDCl_3$). Tetramethylsilane was used as an internal reference. Magnetic field was set at 11.75 Tesla, in 89 mm magnet bore size and 5 mm multinuclear broad band probe (BBO CryoProbe). Spectral data were generated from Bruker TopSpin® NMR software.

### Fourier-transform Infrared Spectroscopy (FTIR)

The chemical functional groups of the isolated compound were determined using Fourier-transform infrared spectrophotometer, Shimadzu IRPrestige-21 (Duisburg, Germany). The wavelength range was recorded between 400 and 4000 $cm^{-1}$. Data were generated by Shimadzu IRSolution software.

### Gas Chromatography-mass Spectrometry (GC-MS)

GC-MS was done using Perkin Elmer AutoSystem™ XL chromatograph with TurboMass™ spectrometer (Waltham, USA). Acetonitrile was used as a solvent. A non-polar capillary column Elite-5MS (30 m × 0.25 mm × 0.25 µm) was the stationary phase. Temperature of the injector port was fixed at 260°C, while that of the oven was started at 75°C and increased up to 280°C. The carrier gas, helium was allowed to flow at 1 mL/min. 1 µL of the sample was injected in a splitting ratio of 1:50. Electron ionization was set at 220°C with the electron energy at 70 eV and the mass scanned at the range of 50–700 *m*/*z*. TotalChrom® software was used to maintain the data.

### Anthelmintic Susceptibility Assay

The anthelmintic susceptibility was evaluated using a tapeworm, *Davainea penetrans* Baczynska, 1914 (family Davaineidae; phylum Platyhelminthes), were dissected out from the intestines of chicken, *Gallus gallus* Linnaeus, 1758. Based on the method of helminth survival assay [18], the worms were treated with the isolated compound in a microbiological incubator. Albendazole at 20 mg/ml was used as an anthelmintic reference. Culture medium was composed of 0.9% neutral phosphate-buffered saline (PBS) and 1% dimethyl sulfoxide (DMSO). The anthelmintic susceptibility was determined from the duration of survival of the worms. The data were presented in means ± standard deviation of the means, and compared by Student's *t*-test, the level of significance taken at a *p* value less than 0.05. Group comparison was performed using one-way ANOVA with Tukey's multiple comparison test in Prism GraphPad 10.4.0 (Dotmatics, California, USA).

### Scanning Electron Microscopy

Tapeworms exposed to the isolated compounds were processed for analysis in scanning electron microscopy according to the standardized procedure for helminth parasites [19]. The worms were washed with PBS and fixed in 10% neutral-buffered formaldehyde at 4°C for 4 h. They were again fixed in osmium tetroxide. The worms were completely dehydrated through increasing grades of acetone. The tissues were stabilized by treating with tetramethylsilane and the solvent was evaporated in an air-drying cabinet maintained at 25 ± 1°C. The specimen surfaces were coated with gold in an ion sputter JFC-1100 (JEOL Ltd., Tokyo, Japan). Micrographs were taken with a scanning electron microscope JSM-6360 (JEOL Ltd., Tokyo, Japan).

## RESULTS

### Isolation and Compound Identification

Soxhlet extraction of *A. oleracea* using hexane gave a low yield with an extractive value of 4.07%. Hexane is a highly non-polar solvent with polarity index of 0.1, for which general low yield was expected. The concentrated and dried samples were whitish amorphous powder. The main compound was obtained as white needle crystals detected by TLC at an $R_f$ value of 0.56 from a mobile phase made up of hexane and ethyl acetate in 9:1 ratio. The resultant fractions at 0.1% ethyl acetate were consistently detected to contain the same compound.

CHNS-O elemental analysis showed the compound is mainly composed of carbon (with a composition of 81.17%), with small amounts of hydrogen (13.05%) and nitrogen (0.7%).

Further chemical analyses established that the compound was lup-20(29)-en-3-ol (Figure 1). APCI-ESI-HRMS mass spectra indicated the compound as a pentacyclic lupane-type triterpene. The mass spectrum of the isolated compound indicated a parent molecular ion [$M^+$] peak at 426 *m*/*z* that corresponds to the molecular formula, $C_{30}H_{50}O$ (Figure 2). The data indicate that the compound was a hydride of lupane.

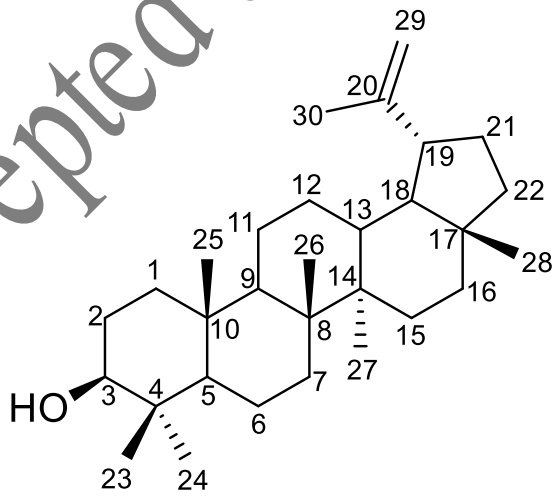


**Fig. 1** Structure of lup-20(29)-en-3-ol.

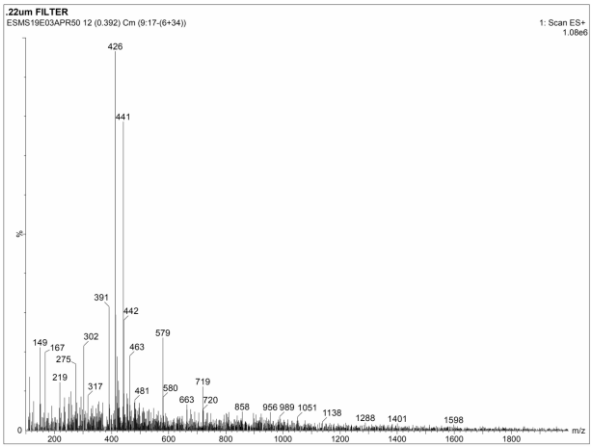


**Fig. 2** APCI-ESI-HRMS mass spectra of a compound isolated from *A. oleracea.*

As described in Figure 3, the $^{1}$H NMR spectrum revealed distinct methyl signals at seven positions, *viz*. δ 1.68, 1.03, 0.97, 0.94, 0.83, 0.79, and 0.76 ppm. The manifestation of two singlet at δ 4.68 and 4.56 ppm for C-29 (2H, H-29a and H-29b), along with that methyl signal at δ 1.68 ppm for proton attached to carbon position 30 (with an integration of three protons) indicated that the isolated compound was a lupane-type triterpenoid. In addition, the observation of a characteristic signal at δ 150.95 and 109.35 ppm (C-20 and 29, olefinic carbon atoms) in its $^{13}$C NMR spectrum (Figure 4) further supported the isolated compound as lupane-type triterpenoid. The singlet signal at δ 79.00 ppm was identified as C-O carbon atom (C-3). A total of 30 carbon signals were observed from the spectrum.

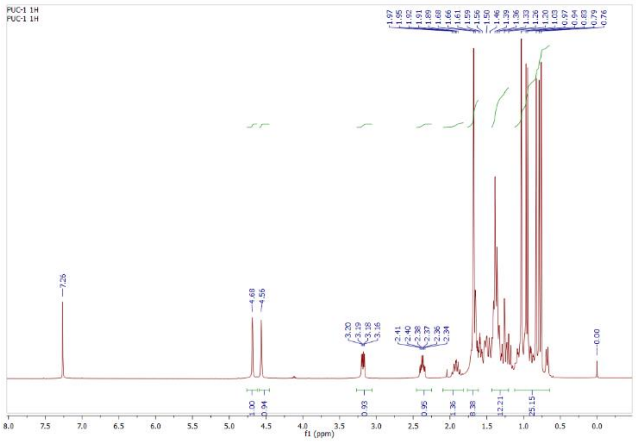


**Fig. 3** $^{1}$H NMR spectrum at 400 MHz in $CDCl_3$ of a compound isolated from *A. oleracea.*

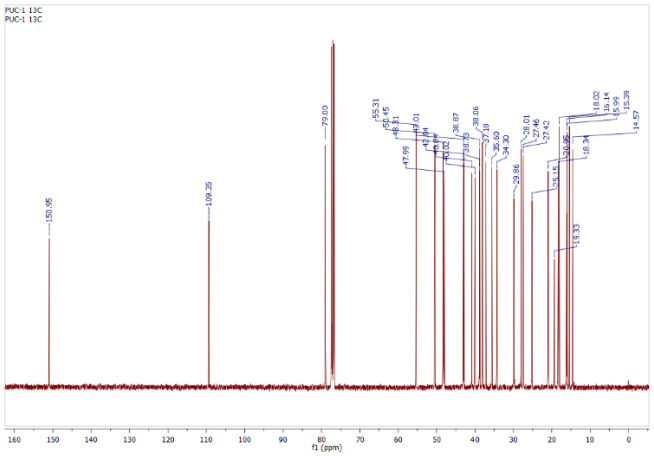


**Fig. 4** $^{13}$C NMR spectra at 100 MHz in $CDCl_3$ of a compound isolated from *A. oleracea.*

Analysis of the IR absorption bands further complemented the structure assigned to lupane-type triterpenoid. FTIR spectra of the compound (Figure 5) showed absorption bands at 752, 941, 1273, 1296, 1435, 1465, 1697, 2846 and 2953 cm$^{-1}$. The observation of a sharp intense peak at 1697 cm$^{-1}$, intense bands at 2953 cm$^{-1}$ and 2846 cm$^{-1}$ indicated the stretching of methylene and methyl parts, respectively.

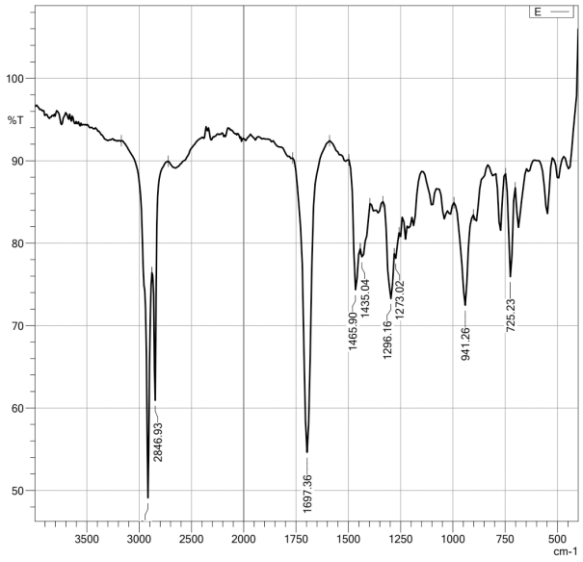


**Fig. 5** FTIR spectra of a compound isolated from *A. oleracea.*

GC-MS chromatograms and spectra indicated that the isolated compound revealed the characteristic fragments of lupane. Comparison of data at the National Institute of Standards and Technology (US Department of Commerce) chemical database indicated that the major peaks at retention times ($R_T$) 30.82, 37.1, 46.47, 52.68, with relative abundance of 31%, 99%, 36% and 35.5% were all detected as lup-20(29)-en-3-ol (Figure 6).

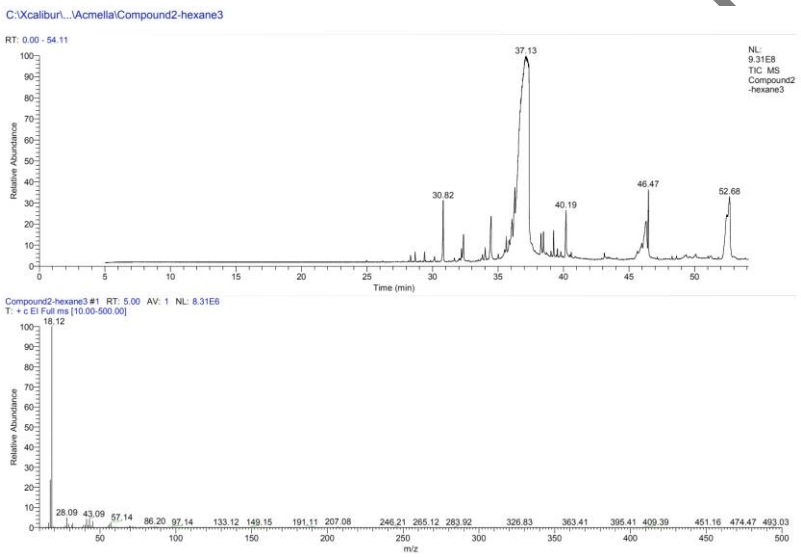


**Fig. 6** GC-MS chromatogram and spectra of a compound isolated from *A. oleracea.* Structure of lup-20(29)-en-3-ol as generated from the NIST chemical library.

## Anthelmintic Susceptibility

The tapeworms collected from fowls could survive over three days in a culture medium (negative control) consisting of PBS with 1% DMSO cultured at 37 ± 1°C in a microbiological incubator at an ambient atmosphere and without food supplementation (Table 1). They were highly susceptible to albendazole at the standard dosage, i.e., 20 mg/mL, with total lethality at 3.27 ± 1.32 h. At corresponding concentrations, *A. oleracea* compound caused complete mortality at 4.29 ± 1.65 h. Multiple comparison of treatment groups using one-way ANOVA showed that both the compound and the drug exhibited highly significant ($p$ <0 .002) efficacy against the negative control group, and that they are more or less equally effective against the parasite model (Figure 7).

**Table 1** Susceptibility of tapeworm to albendazole and lup-20(29)-en-3-ol from *Acmella oleracea*.

| Treatment media | Anthelmintic dose (mg/mL) | Survival time (h) in mean ± SD | *t* value | *t* critical value |
|---|---|---|---|---|
| PBS + DMSO | 0 | 73.29 ± 2.86 | NA | NA |
| Albendazole | 20 | 03.27 ± 0.66 * | 58.45 | 1.81 |
| β-Lup-20(29)-en-3-ol | 20 | 04.29 ± 0.75 * | 57.14 | 1.81 |

*Significantly different at $p < 0.05$ against negative control ($n = 9$); DMSO = dimethyl sulfoxide; NA = not applicable; PBS = phosphate-buffered saline; SD = standard deviation.

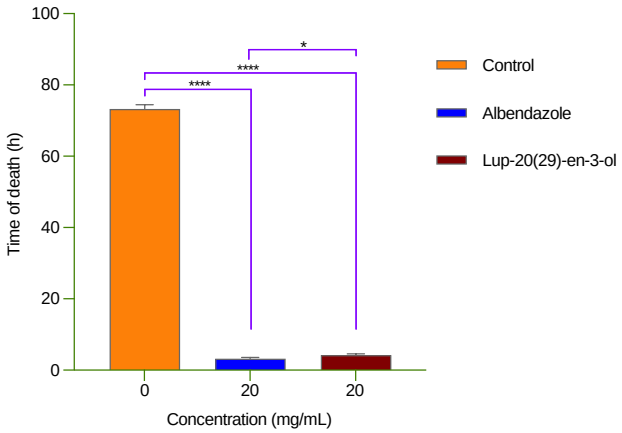


**Fig. 7** Comparison of the survival of *R. echinobothrida* in culture with those treated with albendazole and lup-20(29)-en-3-ol from *A. oleracea* using one-way ANOVA and Turkey's multiple comparison test; $n = 9$; **** $p < 0.0001$; * $p < 0.05$.

### Anthelmintic Effects

Scanning electron microscopy of the tapeworm showed extensive structural distortions and damages all over the body after treatment with *A. oleracea* compound. Severe shrinkage and convolution of the tegument were visible on the entire anterior part including the neck as shown in Figure 8A. The rounder terminal end called the scolex bore two types of parasitic attachments, a circular mouth-like circle called rostellum at the centre of the apex, and four rounded suckers (two of them are visible) on the sides of the scolex. These attachment organs were completely destroyed with loss of the attachment devices called spines and hooks. A barren sucker is shown in Figure 8B, and the same under magnified view (Figure 8C) shows the extent of the damaged sucker. Four white spines remain on the top rim of the sucker and are about to be detached. The tegument of the body segment is uniformly shrivelled with layers of folds instead of smooth and plain surface (Figure 8D). On the mature segments, in addition to the folds, several warts and eruptions of the tegumental tissue are visible (Figure 8E).

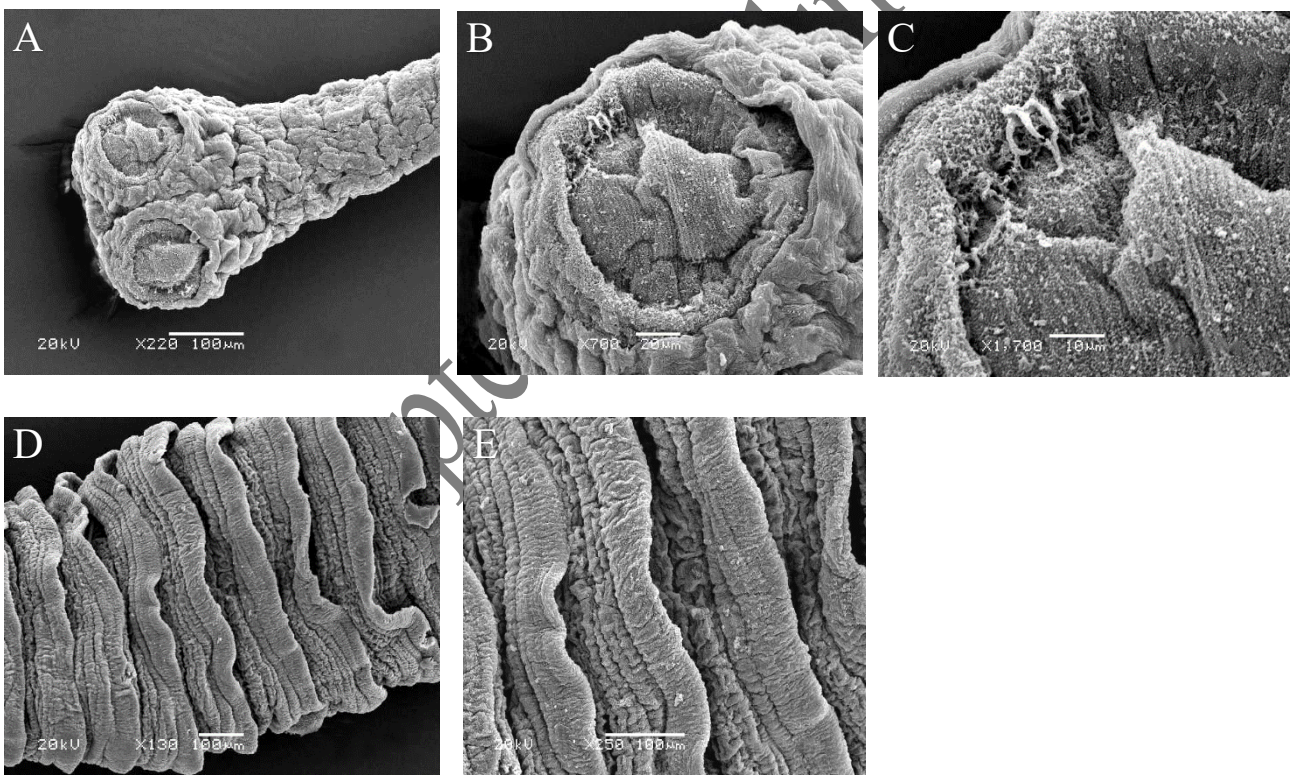


**Fig. 8** Scanning electron micrographs of different body parts of poultry tapeworm treated with a compound isolated from *A. oleracea.* (A) Scolex and neck region showing rostellum (slight depression) at the tip and two oval suckers. (B) One sucker focussed to show removal of the tegument and the spines. (C) Magnification of the same sucker showing almost complete loss of the spines except for four of them (white fang-like structures) on the top. (D) The main body showing a chain of body segments, and the body surface is abnormally folded. (E) Enlarged view of the mature segment indicating heavy tegumental folds and several tissue debris and warts.

## DISCUSSION

*Acmella* species are known to have high contents of fatty acid amides and are particularly rich in various types of N-alkylamides [20-22]. The compounds are the chemical basis of unique physico-chemical properties such as pungent odor and irritating/burning effects in N-

alkylamide-containing plants [23]. In addition, they are the principal bioactive compounds attributed to several pharmacological properties including analgesic, antibacterial, antimutagenic, antioxidant, antiprotozoal, immuno-modulatory, insecticidal and neuroprotective activities [24,25]. In *A. oleracea*, spilanthol and N-isobutyl-2E, 6Z, 8E-decatrienamide were characterized to be the primary bioactive molecules. Spilanthol is specifically shown to be the analgesic compound for which the plant is used in dental treatments, as well as an anaesthetic principle responsible for the botox effect-inducing ability of the plant [20,21,25].

We have previously shown that *A. oleracea* extract exhibits anthelmintic activity against both roundworms and tapeworms [13,17,26]. In searching for the probable anthelmintic principle, we identified several N-alkylamides from several extracts and fractions [26,27]. However, the compounds did not seem have the expected biological activity. We then carried out a full chromatographic scan from which we report here the first isolation of lup-20(29)-en-3-ol from *A. oleracea*. Lup-20(29)-en-3-ol is a pentacyclic triterpene that has been identified from a diverse species of medicinal plants such as *Aegle marmelos, Allanblackia monticola*, *Arbutus unedo*, *Betula platyphylla*, *Bombax ceiba*, *Celastrus paniculatus*, *Crataeva nurvala*, *Emblica officinalis, Hieracium pilosella*, *Leptadenia hastate*, *Tamarindus indica*, *Tipuana tipu*, *Zanthoxylum riedelianum*; as well as in edible fruits such as fig, mango, olive, red grape and strawberry, and common vegetables such as carrot, cucumber, white cabbage, pepper, pea, soy bean and tomato [28,29]. It has been experimentally shown to have an broad range of pharmacological properties such as anticancer, antibacterial, anti-inflammatory, antimalarial and hypocholesterolaemic actions. Studies in animal and human cell lines have indicated that it selectively targets abnormal cells, while avoiding normal healthy cells [30].

Lup-20(29)-en-3-ol's ability to interact with various signalling molecules including nuclear factor kappa B (NFκB), cellular FLICE-like inhibitory protein, EGFR/STAT3, APO-1, Kras, MAPK/ERK, phosphatidylinositol-3-kinase (PI3 K)/Akt, prostaglandin $E_2$ ($PGE_2$), p38/MAPK, RhoA-ROCK1 and Wnt/β-catenin suggests that it has valuable potential in the development of anti-inflammatory and anticancer drugs [29,30]. However, its full cellular actions for drug development are yet to be understood [31]. NMR is considered as the standard technique for identifying the lupane which shows a signature pentacyclic lupane-type triterpene with olefinic protons at δ 4.68 and 4.56 (broad singlets, H-29a and H-29b) and carbons at 150.95 and 109.35 (C-20 and 29), respectively. The proton signals for seven -$CH_3$ groups exhibited a characteristic singlet at δ 1.68, 1.03, 0.97, 0.94, 0.83, 0.79, and 0.76 ppm. Specifically, a chemical shift of δ 2.41-2.34 ppm with an integration of one proton directed to 19β–H is characteristic of a lupane compound [32]. Thus, our data is generally in accord with the published spectral information of lup-20(29)-en-3-ol [33-35].

To our knowledge and available records, there is no report of lupane-type compound as an anthelmintic agent. Ascribing the compound to the anthelmintic property of *A. oleracea*, we demonstrated the high effectiveness against intestinal parasites. Parasitic helminths of poultry are typically innocuous parasites so that their ready availability and easy maintenance in laboratory conditions make them useful test models for anthelmintic susceptibility [18,36,37]. *Taenia* species are characteristic tapeworms with distinctive flat, highly elongated and segmented body, lacking any complex external body parts. Devoid of digestive, excretory and nervous systems, they absorb nutrients and detect their surroundings by their general body covering, the tegument, which is entirely covered with fragile hair-like filaments known as microtriches [38]. Thus, the primary and main target sites of anthelmintics are the tegument so that tegumental disruptions are the defining features of drug effects, which are most conveniently described from electron microscopic studies [36,39].

Benzimidazoles are recognized for their broad-spectrum activities as they are effective against all groups of helminths and are thus considered as the drugs of choice in helminthiasis. The most common benzimidazole, albendazole in a single treatment was shown to cause extensive destruction on the tegument and defacement of the body surface, removal of patches of microtriches, with additional disfigurement of the suckers in *Raillietina echinobothrida* [18]. Extensive collapse of the tegumental layer, mostly in the rostellar complex, along with erosion of microtriches and blistering of tegumental were seen on *Echinococcus granulosus* following combination treatment of albendazole with flubendazole [40]. A combination treatment consisting of albendazole-praziquantel mixture in two species, *E. granulosus* and *Mesocestoides corti*, induced pronounced deformity in the scolex including shrivelling of the suckers, dislodgement of the spines, constriction of the tegument and truncation of the microtriches [41,42]. *R. echinobothrida* exposed to praziquantel caused withering and corrugation of the tegument, loss of microtriches, with damages on the scolex including distortion of the suckers and degeneration of the spines on the suckers [27]. The observed structural changes in the tapeworm after exposure to lup-20(29)-en-3-ol thus indicate the general patterns of anthelmintic effects.

## CONCLUSION

A pentacyclic lupane-type triterpene was isolated from the hexane extract of *A. oleracea*. $^{1}H$ and $^{13}C$ NMR spectra gave the chemical characteristics of lup-20(29)-en-3-ol. GC-MS data confirmed the chemical identity. The plant was already established to have anthelmintic activity so that the compound was tested for antiparasitic activity against a poultry tapeworm. The compound effective killed the parasites *in vitro*, even at the lowest concentration tested. Scanning electron microscopy ascertained the detrimental anthelmintic effects on the tapeworms. Our findings present evidence for lup-20(29)-en-3-ol as a potential lead molecule in anthelmintic development, in addition to its other biological effects. Further pre-clinical and clinical studies are warranted to understand the detailed pharmacological potential of the compound.

### Declaration of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.


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