

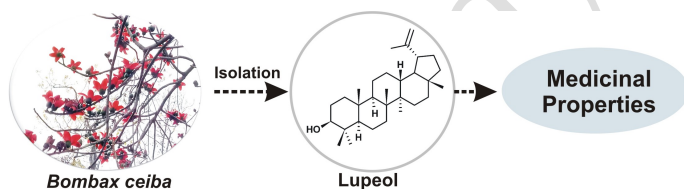
Medicinal Properties of a Naturally Occurring Pentacyclic Triterpenoid Lupeol

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Lupeol is a 6-6-6-6-5 fused pentacyclic lupane type triterpenoid present in different plants. Various medicinal properties of lupeol have been reported for the treatment of different types of cancer, tumor, ulcer, diabetes, various infectious diseases, cardiovascular diseases, wounds, arthritis, chronic inflammation etc. In this review, the medicinal properties of lupeol has been discussed. A useful procedure for the isolation of lupeol 1 from the dried outer bark of *Bombax ceiba* commonly known as "Shimul" (in Bengali) and its detailed characterization has also been reported.



Keywords: Triterpenoid, lupeol, *Bombax ceiba*, biological activity

1. Introduction

The trees are the rich and renewable source of various kinds phytochemicals like polyphenols, terpenoids, flavonoids, carotenoids, xanthanoids, alkaloids, fatty acids etc.^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17} Triterpenoids containing multiple of isoprene units (C₅) are the major plant secondary metabolites, bio-synthesized in plants via complex enzymatic pathway from its biosynthetic precursor squalene or oxidosqualene. Lupeol is a monohydroxy lupane-type 6-6-6-6-5 fused pentacyclic triterpenoid. During the last few decades triterpenoids have gained tremendous research interest in various fields of science due to their (i) nontoxic nature, (ii) natural availability, (iii) nanometric lengths, (iv) amphiphilic character, (iv) bio-compatibility, etc. The molecule lupeol has various medicinal activities for the treatment of chronic diseases like cancer, tumor, ulcer, diabetes, various infectious diseases, cardiovascular diseases, wounds, arthritis, chronic inflammation etc.^{18,19,20,21,22,23,24,25,26,27,28} which have been reported in this review. A useful procedure for the isolation of lupeol from the outer bark of *Bombax ceiba* and its characterization by ¹H-NMR, ¹³C-NMR, DEPT 90, DEPT 135, FTIR and Mass Spectroscopy have also been described.

2. Biological activity of lupeol

Lupeol has been shown to exhibit various pharmacological activities. Lupeol has been demonstrated to have tremendous medicinal activities like antiangiogenic, antiulcer, antioxidant, hepatoprotective, cardioprotective,

chemopreventive activity, antiinflammatory, antimicrobial activities, anti-arthritic activity, antiprotozoal activity, anti-diabetic activity, nephroprotective activity, anti-tumor, antiacne activity etc. (Figure 1, 2 and 3).

2. 1 Anti-inflammatory activity

Anti-inflammatory activity of lupeol has been studied extensively under in vitro conditions and in animal models. S. Singh and co-workers have shown oral or intraperitoneal administration of the derivatives of lupeol to have potent anti-inflammatory activity in the year 1997³⁰. Treatment with lupeol was found to reduce the overall inflammation in the lungs hasd been shown in the year 2001 by Geetha et. al. N. K. Andrikopoulos et. al have demonstrated, lupeol has also been to modulate several number of molecules which directly or indirectly play a crucial role in the anti-inflammatory activity in the year 2003.^{29,30} The anti-inflammatory activity of lupeol was observed to be equal to a well-known anti-inflammatory agent dexamethasone has been demonstrated by J. F Vasconcelos and co-workers in the year 2008.³¹

2. 2 Hypotensive Activity

Hypertension is a kind of disease, if left untreated it affects various organs of human body. It is known as silent killer as it does not show any significant symptoms in the human body. Hypertension may cause cardiac disorders, stroke, brain hemorrhage, renal failure and vision loss etc. Hypertension has affected and killed a large number of global

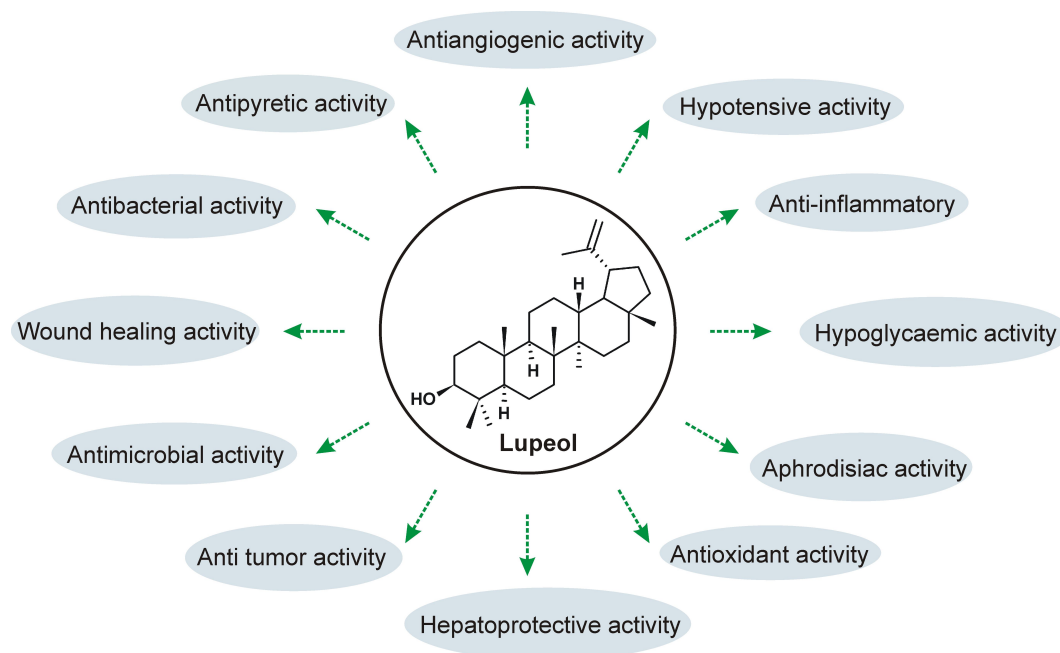


Figure 1: Schematic presentation of biological activities of lupeol

population in the world. Drugs used for hypertension treatment are very expensive. In the year 1999, 2003 R. Saleem and co-workers have demonstrated in vivo study on animals intravenous or oral administration of lupeol along with a dimeric glycoside shamimin possess potent hypotensive activity by lowering blood pressure.^{34,32}

There are several reports in the literature on the anti-diabetic activity of triterpenoids and various plant extracts. In 2013, C. J. Bhabasari and co-workers have shown lupeol is very effective to cause significant hypoglycemic or hypolipidemic effects on diabetic rats by lowering the total cholesterol and triglyceride level.³⁶

2.3 Cardioprotective activity

In vitro investigations with lupeol and its derivatives has been shown to have hypotensive activity and cardio-protective effects to prevent cardiovascular diseases and cardiac disorder as demonstrated against low density lipoprotein (LDL) oxidation by N. K. Andrikopoulos et. al in the year 2003.³³ Moreover, supplements of lupeol has been shown to be very much effective against the cardiac injury caused by drugs when used for the treatment of cancer and auto-immune disorders which has also been demonstrated by R. Saleem and co-workers in 2003.³⁴

2.4 Antimicrobial and antibacterial activity

There are many reports in the literature depicting the antimicrobial and antibacterial activity of triterpenoids and various plant extracts. Plant extracts of *Bombax ceiba* containing a potent bio-active triterpenoid lupeol showed strong antibacterial activity as examined by agar disc diffusion method. The activity was comparable to the standard antimicrobial drugs amikacin and piperacillin which has been shown in the year 2004 by R. Phulan and co-workers.³⁵

2.5 Anti-diabetic activity

2.6 Anticancer activity

Biological studies carried out by W. N. Setzer et. al in 2003 shows that lupeol shows antineoplastic effect³⁷ and poses antiproliferative activity as examined against various types of cancer cell lines by K. T. Liby and co-workers 2007.^{38,39} Under in vitro and in vivo systems, Lupeol has been shown to exhibit strong anti-mutagenic activity.⁴⁰ M. Saleem and co-workers has demonstrated in 2004, lupeol inhibits the chemically induced DNA damage. It is reported that lupeol significantly inhibits the activity of ornithine decarboxylase (ODC) protein, a well-known biomarker for tumor promotion.⁴¹ Lupeol also inhibits the growth of human malignant melanoma cancer cells and leukemia cells without affecting normal human melanocytes which has also demonstrated by Y. Aratanechemuge and co-workers in 2002. Recently, studies carried out by K. Hata et. al in 2004 have shown the structure-activity relationships of lupeol in various human cancer cell lines.^{42,43} In vitro experiments, showed synergistic effects of lupeol with chemotherapeutic drug cis-platin, resulting in chemo-sensitization of head and neck carcinoma cell lines (HNSCC) with better activity in 2007 by T. K. Lee et. al.⁴⁴ Lupeol has been demonstrated to suppress the migration and invasion in human osteosarcoma U-2 OS cells in 2019 by M. J. Husa and co-workers (Figure 3).⁴⁵

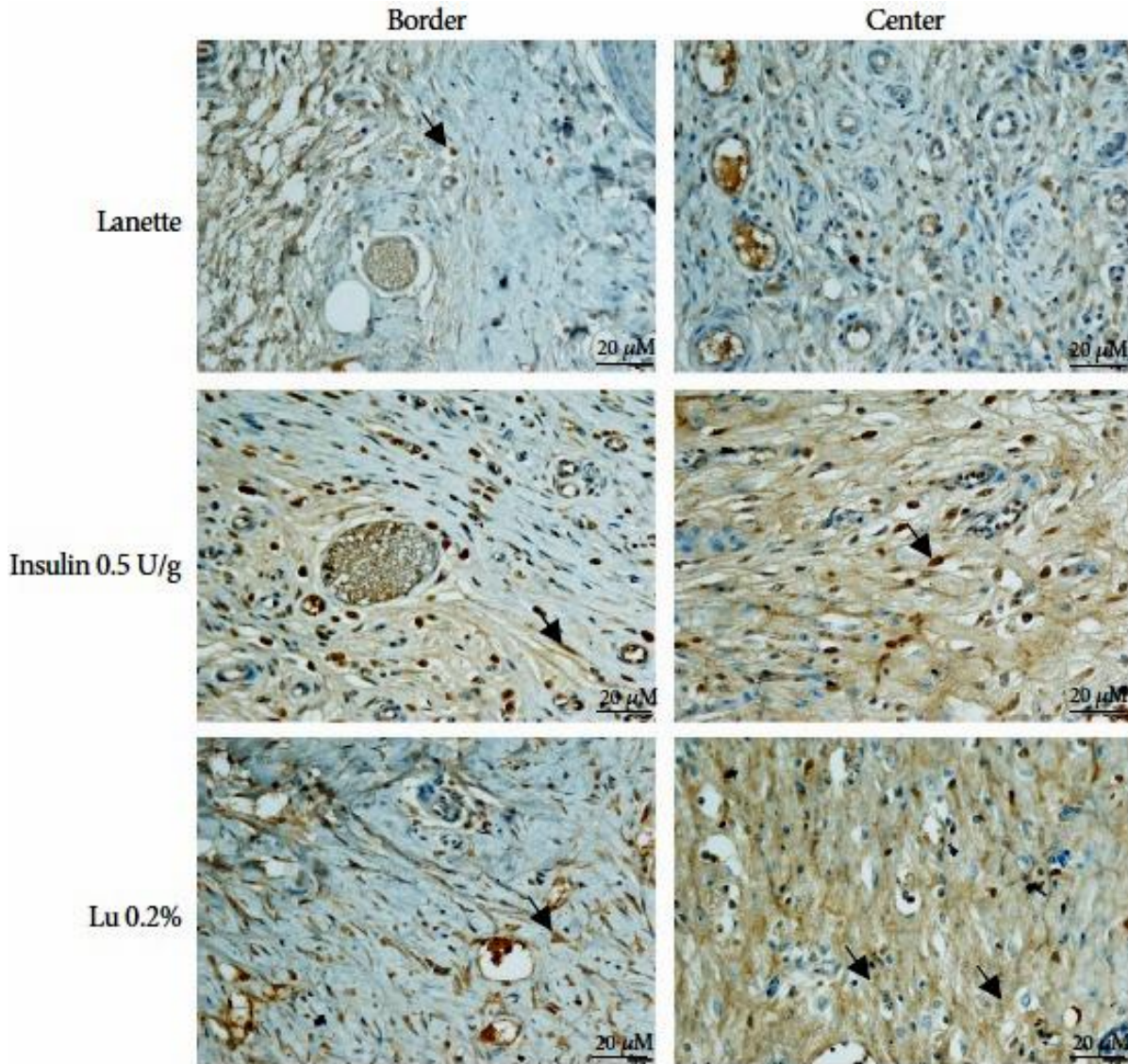


Figure 2: Photomicrography of the immune-staining and immune-labeled area (μm^2) for collagen III (g, h) in the border and central region of rats' hyperglycemic wounds treated with Lanette, insulin 0.5 U/g, or lupeol 0.2% for 14 days. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. Lanette group. $\#p < 0.05$ vs. insulin group, using ANOVA followed by the Newman-Keuls test. Bar represents 20 μm . Black arrows indicate antibody staining against collagen III. Lu 0.2% = lupeol 0.2%. (Adapted from Ref. 27)

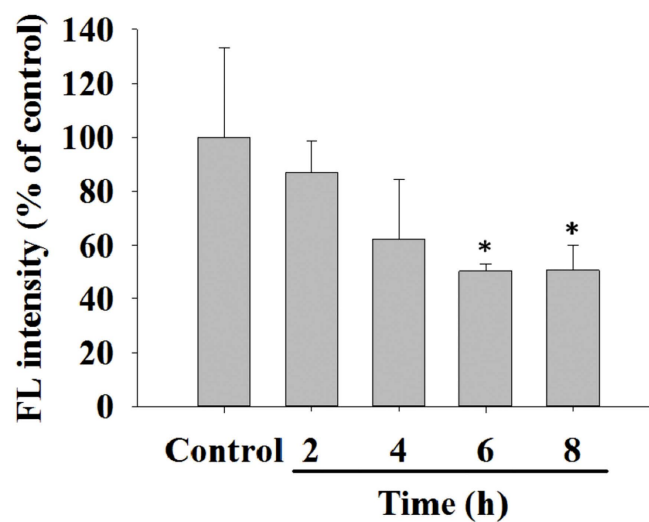
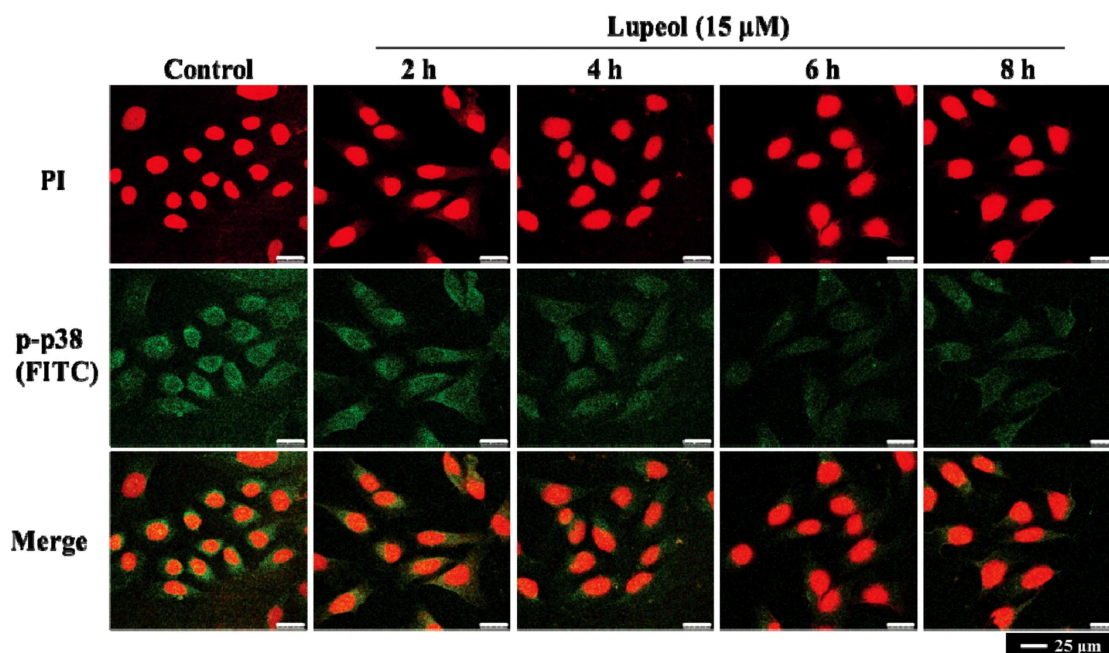


Figure 3: Lupeol affected the levels of associated proteins in cell migration and invasion of U-2 OS cells. Cells (1×10^6 cells/dish) were treated with lupeol (15 μM) for 2, 4, 6, and 8 h. Expressions were estimated by western blotting. Or cells were cultured and treated with lupeol (15 μM) for 2, 4, 6, and 8 h and examined the p-p38 levels and fluorescence intensity by confocal laser microscopy. (Adapted from Ref.37)

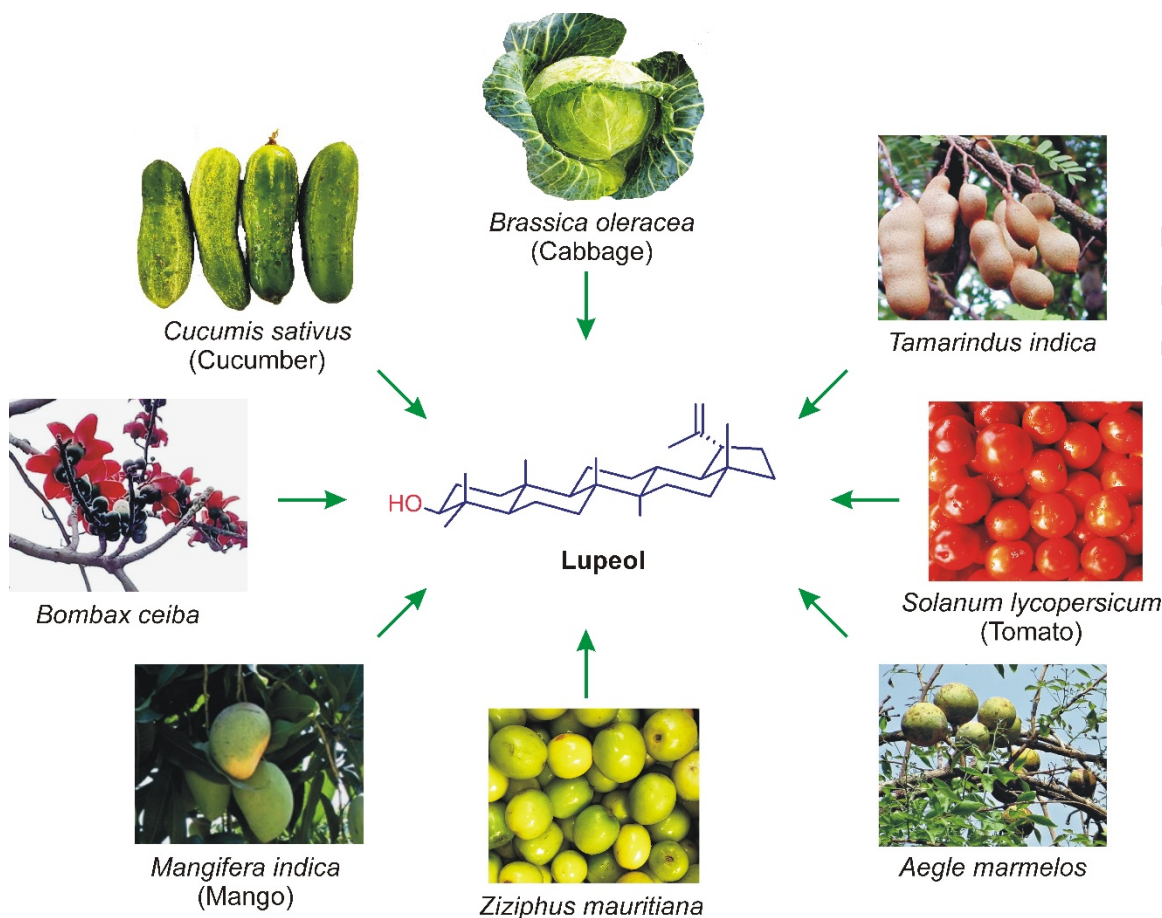


Figure 4: Schematic presentation of natural sources of lupeol

3. Source of lupeol

Presence of lupeol has been reported in different plants including many vegetables such as white cabbage, cucumber (*Cucumis sativus*), Carrot (*Daucus carota*), Licorice (*Glycyrrhiza glabra*), pepper (*Capsicum annuum*), Ivy gourd (*Coccinia grandis*), Bitterroot (*Apocynum cannabinum*), Soya_bean (*Glycine max*), tomato (*Lycopersicon esculentum*), aloe vera etc⁴⁶. It is also present in various fruits like mango, date Palm (*Phoenix dactylifera*), guava (*Psidium guajava*), grapevine (*Vitis vinifera*), red grapes, olive, elm plant, strawberry etc⁴⁶. L. J Swift et. al. in the year 1942 isolated lupeol from *Osage orange*⁴⁷. In 1970, J. Blair et. al isolated lupeol from the bark of *Heritiera utilis* (*Tarrietia utilis*)⁴⁸. In 1994 R. Anand et. al isolated from *Crataeva nurvala*⁴⁹. A. Fernández et. al isolated lupeol from *Pimenta racemosa* in 2001⁵⁰. R. B. Agarwal et. al isolated from *Strobilanthes callosus* and *Strobilanthes ixiocephala* roots in 2003⁵¹. S. Bani et. al isolated lupeol from *Crataeva religiosa* in 2006⁵². S. Imam isolated lupeol from *Tamarindus indica* in 2007⁵³. In 2009, M. Na et. al isolated lupeol from *Sorbus commixta*⁵⁴. In the year 2011, V. Saratha and co-workers isolated lupeol from *Calotropis gigantea* latex⁵⁵. D. Pitchai et. al isolated lupeol from *Elephantopus scaber* in 2014⁵⁶. It is also present in various parts of many medicinal plants such

as *Bombax ceiba*, *American ginseng*, *Celastrus paniculatus*, *Allanblackia monticola*, *latex of Leptadenia hastate*, bark and stem of *Butea monosperma*, *Lactuca indica*, *Himatanthus sucuuba*, *Zanthoxylum riedelianum*, *Himatanthus drasticus*, etc.^{57,58,59,60,61} (Figure 4). Most of the plants described here are used in our daily life and are medicinally important.

Among the above plant resources, *Bombax ceiba* (Shimul) has a straight tall trunk and its leaves are deciduous in winter. Its flowers are red in colour with thick five petals. It produces a capsule which, when ripe, contains white fiber-like cotton. Its tall trunk bears spikes to prevent attacks by animals. *Bombax ceiba* grows in plenty in Midnapore, West Bengal, India. Presence of lupeol has been reported in the bark of *Bombax ceiba*. From the ancient time different parts of *Bombax ceiba* is being used as traditional medicine in Ayurveda and unani system for the treatment of diarrhea, chronic inflammation, fever, dysentery, influenza, pulmonary tuberculosis, catarrhal affection and ulceration of the bladder and kidney and also as tonic.⁶² In modern time, various parts of *Bombax ceiba* also have been reported for potential biological activities like hypotensive and hypoglycaemic, antiangiogenic, analgesic, antiulcer, antioxidant,

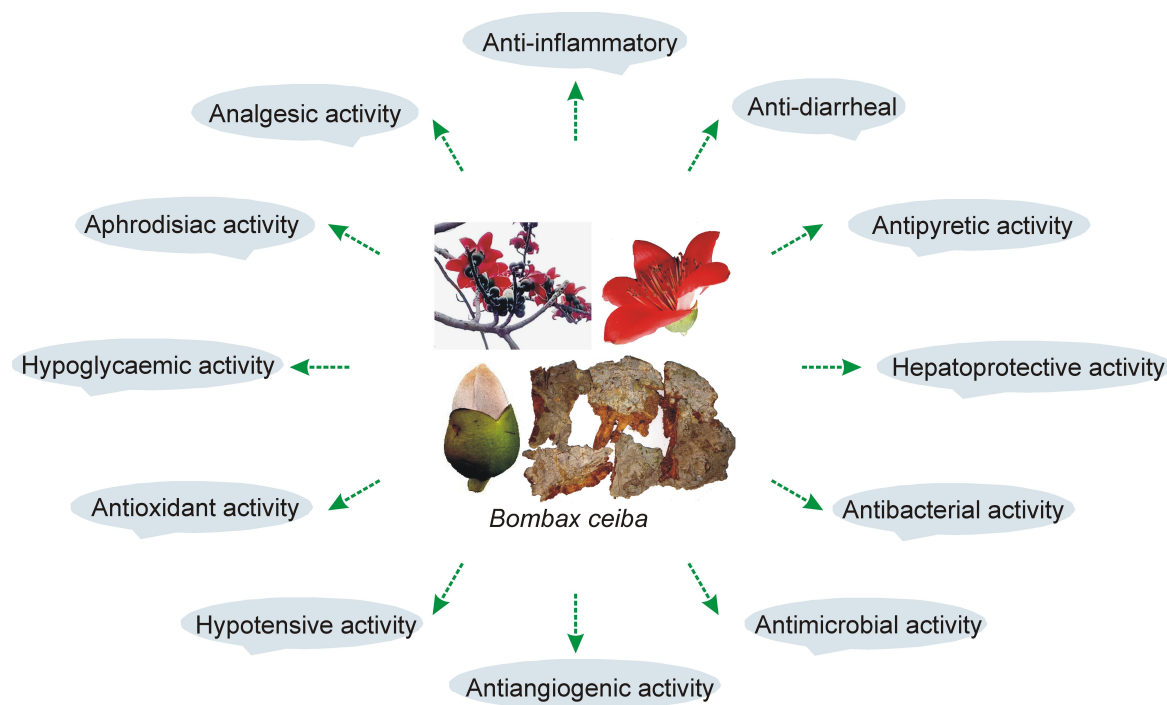
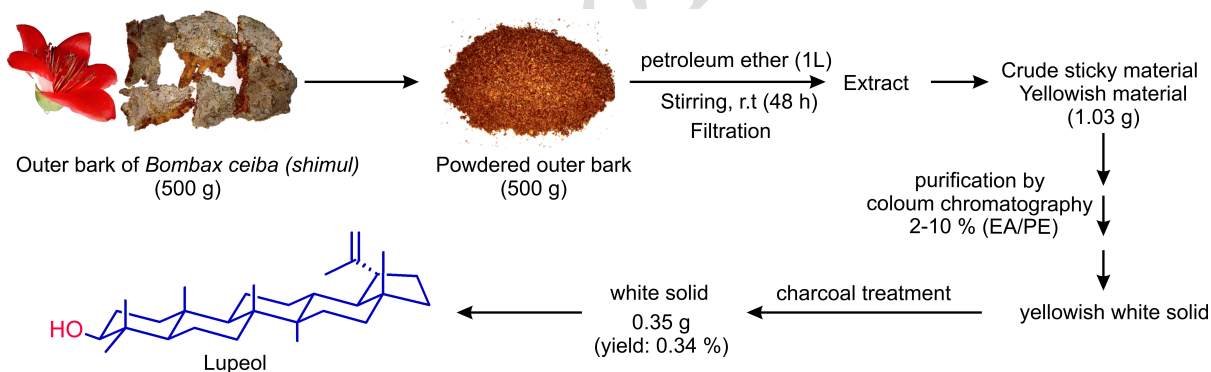


Figure 5: Schematic presentation of biological activities of *Bombax ceiba*

hepatoprotective, antimicrobial activities, healing wounds etc (Figure 5).

river side, Paschim Medinipur, West Bengal, India, in February 2019.



Scheme 1: Schematic presentation of isolation of lupeol from the outer bark of *Bombax ceiba*

3. Experimental Section

3.1 General Experimental Procedures

All commercial grade solvents used for the extraction, isolation and purification were distilled prior to use. Spectral characterizations like ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100MHz respectively, on a Bruker NMR spectrometer. HRMS was obtained with a The Agilent 6230B TOF. FTIR was obtained with Perkin Elmer.

3.2 Plant Material

The fresh outer bark of *Bombax ceiba* (*shimul*) sample used in this study was collected from Kangshabati

3.2 Isolation and Purification of Lupeol

Sun dried finely powdered outer bark of *Bombax ceiba*, commonly known as *shimul* (in Bengali) (500 g) was stirred magnetically at room temperature with petroleum ether (1 L) for 48 h and then filtered via filter paper. The volatiles were removed under reduced pressure to afford a yellowish sticky material (1.03 g). The crude extract was purified by successive column chromatography (2 times, Sigel, 100–200 mesh, 1.2×15 cm) using 2–10% ethyl acetate/petroleum ether as the eluent. The product appeared as a white solid (0.350 g, 0.34 % yield) (scheme 1). R_f : 0.3 (10% ethyl acetate/ petroleum ether). White powder, MP = 210 – 212°C.

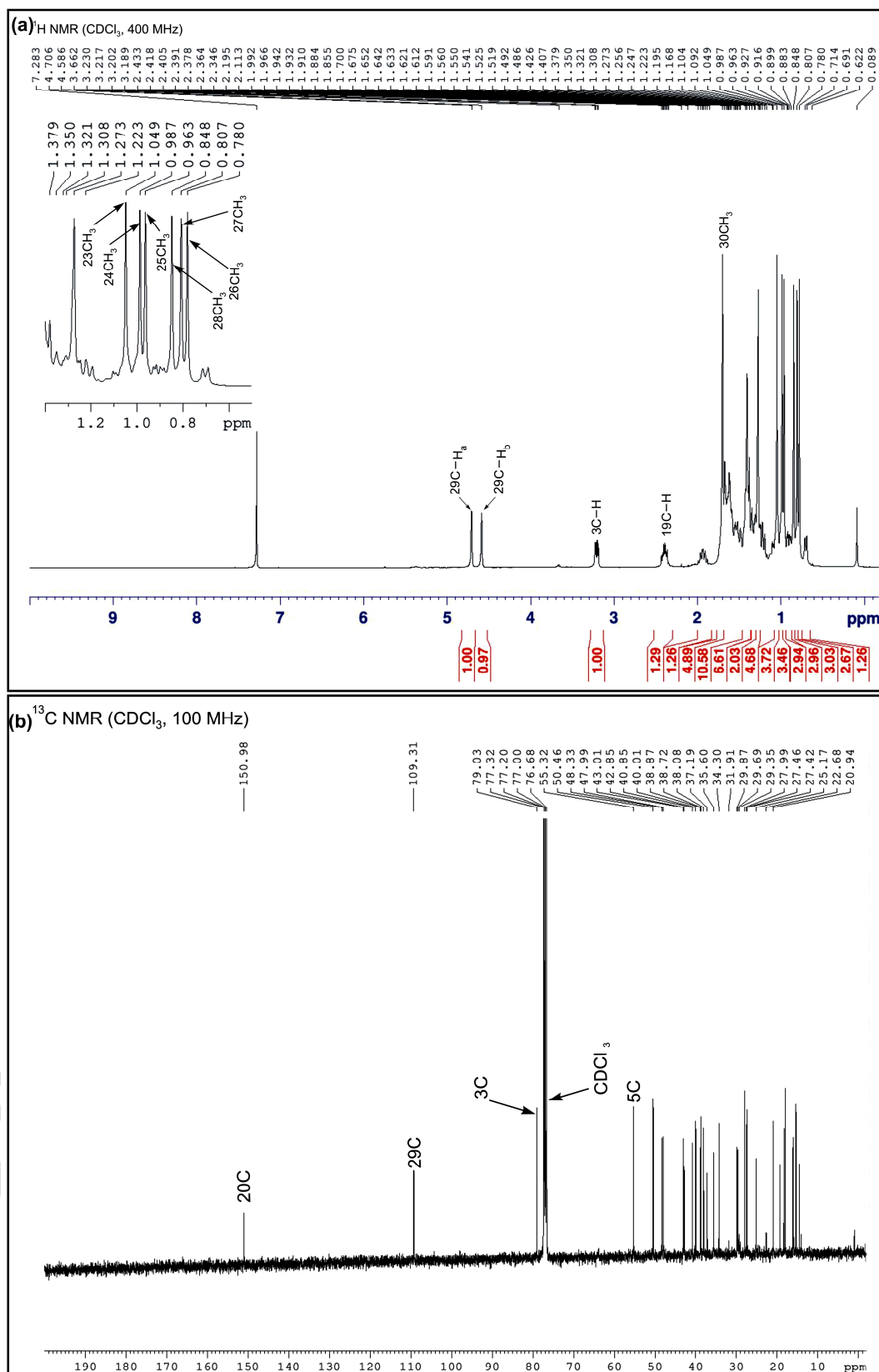
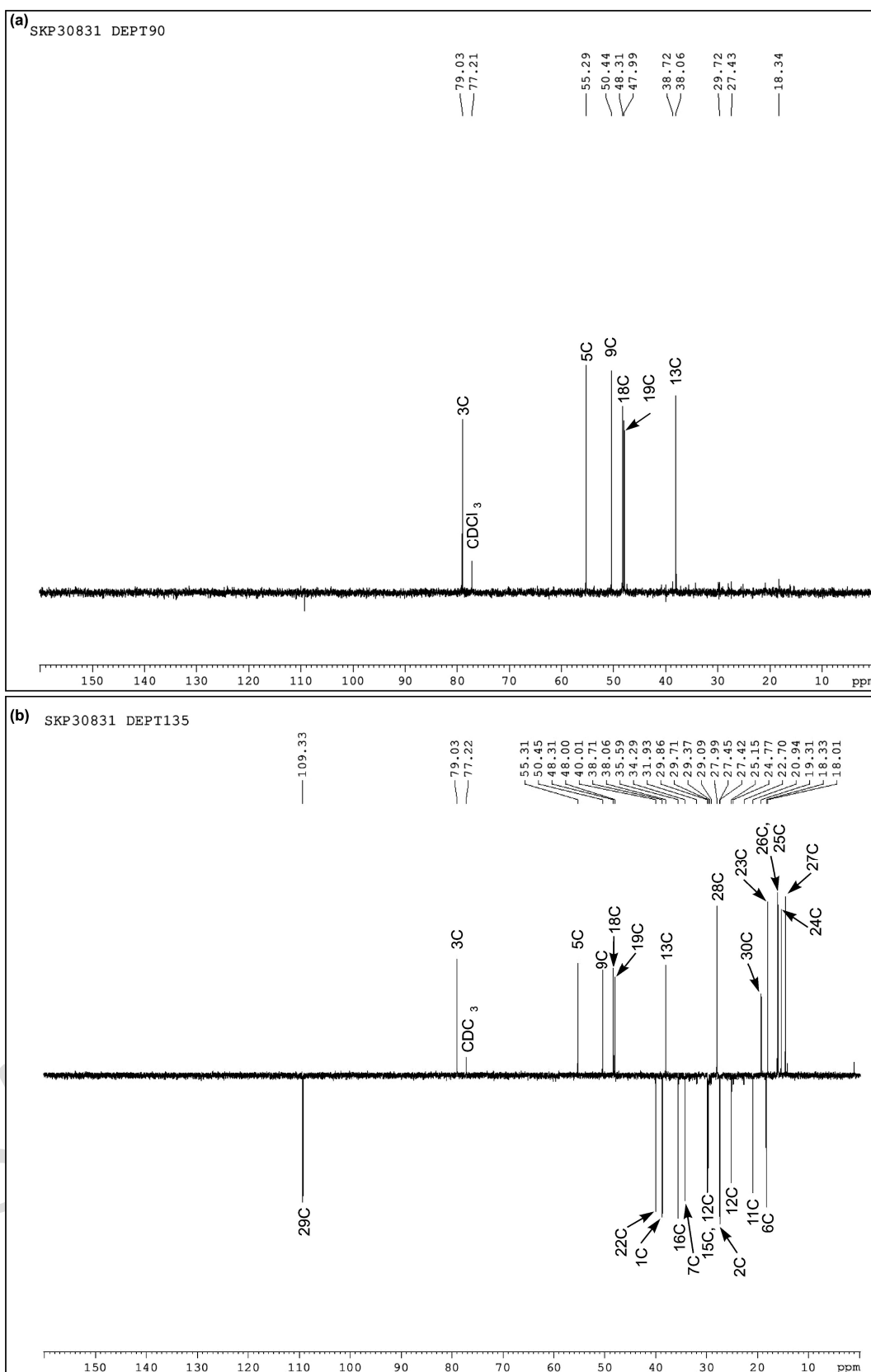


Figure 6: (a) ^1H NMR (400 MHz, CDCl_3) and (b) ^{13}C NMR (100 MHz, CDCl_3) spectrum of lupeol



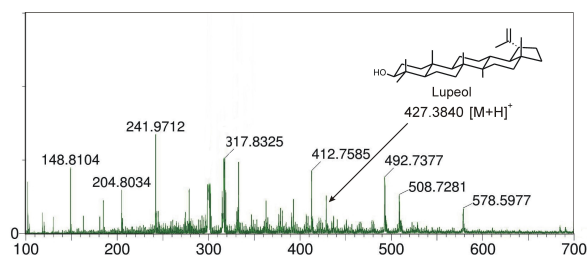


Figure 8: ESI-MS spectrum of lupeol 1

3.3 Structural Characterization

The structure of the isolated compound was established on the basis of different spectroscopic techniques like HRMS, ^1H NMR, ^{13}C NMR, DEPT 90, DEPT 135 and FTIR.^{63,64,65,66} Lupeol 1, molecular formula $\text{C}_{30}\text{H}_{50}\text{O}$ has been established by HR-MS. ^1H NMR (400 MHz, CDCl_3): δ 4.69 and δ 4.57 (each 1H, s), δ 3.20 (1H, dd), δ 2.40 (1H, m), δ 1.70 (3H, s), δ 1.04 (3H, s), δ 0.98 (3H, s), δ 0.96 (3H, s), δ 0.84 (3H, s), δ 0.78 (3H, s), δ 0.74 (3H, s) (Figure 6a). From ^1H NMR spectrum it is evident that the molecule has seven “- CH_3 ” groups having δ value in the range 0.78 - 1.70 ppm and two olefinic protons attached with having the δ value 4.69 and 4.57. ^{13}C NMR (CDCl_3 , 100 MHz): δ 38.8, 27.5, 79.0, 38.7, 55.3, 18.0, 34.3, 40.8, 50.4, 37.2, 20.9, 25.2, 38.1, 42.8, 27.4, 35.6, 43.0, 48.3, 48.0, 150.9, 29.8, 40.0, 27.9, 15.3, 16.1, 15.9, 14.5, 18.0, 109.3, 19.3 (Figure 6b). The ^{13}C spectrum clearly indicated the presence of 30 carbon atoms in the molecule. To further elucidate the structure of the molecule, DEPT 90 and DEPT 135 experiments were carried out. DEPT 90 (CDCl_3 , 100 MHz): δ 79.0, 55.3, 50.4, 38.1, 48.3, 48.0. DEPT 90 clearly indicated the presence of six “- CH ”s in the molecule. (Figure 7a); DEPT 135 (CDCl_3 , 100 MHz): δ 109.3, 27.5, 18.0, 34.3, 20.9, 25.2, 27.4, 35.6, 29.8, 40.0, 37.2 (Figure 7b). DEPT 135 indicated the presence of one olefinic carbon (= CH_2) which is attached with quaternary carbon as evident from DEPT 90 and ^{13}C NMR. Negative peaks observed in DEPT 135 clearly indicated the presence of eleven “- CH_2 ” in the molecule. FTIR (ν , cm^{-1}): 3310 (w), 3066, 2925 (s), 2853 (s), 1713, 1638, 1453, 1381, 1188, 1038, 980, 877, 802 (Figure 5). The FTIR spectrum confirmed the presence of the -OH group (3310 cm^{-1}) and C=C (1638 cm^{-1}). The NMR data obtained for the compound are comparable to those previously reported in the literature. HRMS: calculated for $\text{C}_{30}\text{H}_{50}\text{O}$ 426.3861; obtained 426.3846.

4. Conclusion and Outlook

Here in we have reviewed various natural sources and pharmacological activities of lupeol. Moreover, a useful method for the isolation of lupeol from the dried outer bark of *Bombax ceiba* has been described. The detail structural characterization of lupeol has been carried out by spectroscopic methods like ^1H NMR, ^{13}C NMR, DEPT-135, FTIR and GCMS. The molecule has a lipophilic triterpenoid backbone with one secondary hydroxyl (-OH) situated at one

extreme end of the molecule making it useful as a *molecular functional nano-entity* in the design of advanced functional materials and nano-biotechnology.

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