Isolation and Characterization of Lupeol from the Stem of *Tapinanthus globiferus* (A. Rich.) and its Antimicrobial Assay

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**ABSTRACT:** Lupeol, a pentacyclic triterpenoid, was isolated from hexane and ethyl acetate solvent system. In antiquity, the stem and leaf infusion of *Tapinanthus globiferus* has been used ethno-medicinally as a remedy for stomach ache, diarrhea, dysentery, and wounds. Lupeol isolation from this species was carried out by column chromatography after concentrating the crude extract using a rotary evaporator, and the structure was determined by analysis of the isolate by IR, 1C NMR, 1H NMR, HSQC, and HMBC spectral analysis as well as comparison with reported data. This is the first isolation of lupeol from the stem of this species.

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*Tapinanthus globiferus* is a mistletoe of the family Loranthaceae. It grows parasitically on trees and shrubs, nearly all the Loranthaceae family grow in the tropics. It is a woody, spreading shrub with blackish, smooth stems that look rough due to lenticels. The previous studies conducted which includes antibacterial effect (Emaikwu et al., 2019) antioxidant effect (Cork et al., 1998) and antihypertensive studies via reduction of LDL and triglycerides. It was also reported that the leaf decoction from this plant group is used for the treatment of hypertension, ulcers, epilepsy, diabetics, weakness of vision, and for promoting muscular relaxation before delivery. The objective of this paper is to report the isolation of lupeol from hexane-ethyl acetate extracts of birdlime (*Tapinanthus globiferus*) because Lupeol has demonstrated several intriguing pharmacological activities including anti-angiogenic, anti-microbial, anti-proliferative, anti-inflammatory, and cholesterol-lowering (Avin et al., 2014; Saleem, 2009; Gallo and Sarachine, 2009). Owing to its non-toxicity to normal cells and tissues, chemoprevention with lupeol is relatively a new avenue of oncology (Chaturvedi, 2008; Palanimuthu et al., 2012).

**MATERIALS AND METHODS**

*Plant collection:* Fresh stem samples of *Tapinanthus globiferus* growing on *Terminalia catappa* were collected from Samara in Zaria, Nigeria, in the month of July-August 2017 and sundried, the sundried stem was pulverized using mortar and pestle.

*Extraction:* 500g of the pulverized stem was measured into a clean Winchester bottle and 1.5 liters of hexane was introduced and shaken intermittently and then filtered after 48 hours, the same was repeated for ethyl acetate. Extracts were concentrated to one-third of the original volume in vacuo using a rotary evaporator at about 42°C, this yielded 1.2g (0.24%) of crude n-hexane extract, 1.2g (0.24%) of crude ethyl acetate extract and 2.5g (0.5%) of methanol extract. These were then subjected to bioassay studies (Sofohora, 2008) and after subsequent isolation, the isolated compound V39 was subjected to several spectral analyses.

*Isolation and Purification:* The hexane and ethyl acetate extract of *Tapinanthus globiferus* were dissolved in a minimum amount of ethyl acetate and hexane solvent system 9:1, preabsorbed in silica gel and subsequently loaded onto a column packed with silica gel. Normal phase adsorption chromatography was carried out with gradient elution using different solvent systems of hexane-ethyl acetate (10.0, 9.5:0.5, 9.0:1.0, 8.5:1.5, 8.0:2.0, 7.5:2.5, 7.0:3.0, 6.5:3.5, 6.0:4.0, 5.5:4.5, 5.0:5.0) in an increasing order of polarity. The solvent system in the ratio 9:1 was used as the basis of the TLC monitoring of the column chromatography which gave an isolate V39 and also...
gave a single homogenous spot on TLC with the solvent system Hexane / Ethylacetato (9:1). This compound, tagged V39, appeared as white needle-like crystals and was subjected to several spectral analysis.

**Spectral Analysis:** Several spectroscopic techniques were used to elucidate the structure of the isolate V39, including IR, 2D $^1$H NMR, $^{13}$C NMR, HSQC, and HMBC techniques. The IR spectrum was recorded on FTIR-400s (Shimadzu) in CCl$_4$ at Ahmadu Bello University multipurpose laboratory, Zaria, the NMR spectra were recorded on a Bruker ADVANCE (400MHZ for $^1$H, and 125MHz for $^{13}$C) in deuterated chloroform (CDCl$_3$). The spectral analysis and comparison with reported data led to the proposition of the structure of V39 as lupeol, a pentacyclic triterpenoid (Figure 1).

**RESULTS AND DISCUSSION**

**Spectral analysis of the isolate V39:** To obtain some useful information on the bioactive compound isolated via the different chromatographic technique i.e TLC and column chromatography, detailed spectral analysis viz FTIR, 2D $^1$H NMR, $^{13}$C NMR, HSQC, and HMBC studies were taken. The presence of a hydroxyl functional group and an olefinic moiety which show their presence in the spectrum at 3388.2 and 1658.7 cm$^{-1}$ respectively were identified.

The IR spectrum of V39 (Fig.1) showed a very intensely broad absorption frequency peak at 3388.2 cm$^{-1}$ typical of the O-H bond stretch of the hydroxyl group. A fairly intense band at 2922.2 cm$^{-1}$ that can be assigned to an aliphatic C-H stretch was observed. The C=C vibrations were observed at 1658.7 cm$^{-1}$ as an intense band and corresponding C-H vibrations of the unsaturated portion at 1013.8 cm$^{-1}$ (Table 1.0). From the proton NMR spectrum shown in figure 3.0, 42 proton signals were observed. The signals at 0-2.0 ppm are due to overlapping methine (CH), methylene (CH$_2$) and methyl (CH$_3$) protons. The signal at 3.64 ppm is the characteristics of oxymethylene proton while the signals between 5 and 6 ppm are due to olefinic protons. These three regions of signals are characteristic of steroidal nuclei.

The carbon thirteen NMR spectrum (Fig.2) revealed 30 carbon signals. The signals from 0-55 ppm correspond to methine (=CH), methylene (=CH$_2$) and methyl (-CH$_3$) carbon at 79.17 ppm. The deshielded signal at 79.17 is characteristic of an oxymethine carbon. The signals at 109.47, 23.81 and 151.00 ppm are typical of olefinic carbon atoms. These regions are also typical of steroidal nuclei. These carbon signals in ppm were recorded and compared with available literature data (Tables 2). The HSQC spectrum (Fig.4) shows the correlation between carbon and proton signals via a single bond coupling. Some important correlations are those of the carbon signals at

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**Fig 1.** Displays the infra-red spectrum of lupeol isolated from the bark of *Tapinanthus globiferus*.

**Fig 2.** $^{13}$CNMR of V39

**Fig 3.** 2D $^1$HNMR of V39
Isolation and Characterization of Lupeol from the Stem

0.68 ppm, the carbon signal at 79.22 ppm correlates with the proton signal at 3.14 ppm and the correlation between the carbon signal at 109.67 ppm and the proton signal at 1.04 ppm. The HMBC spectrum (Fig. 5) revealed observable correlations which included the cross-peaks between proton peaks at 1.67 ppm and various carbon signals at 151.49, 109.67 and 48.35 ppm. The HMBC also showed a correlation between proton peak 0.95 ppm and carbon signals at 79.33, 55.56 and 15.57 ppm. The spectral analysis and comparison with reported data led to the proposition of the structure of V39 as lupeol, a pentacyclic triterpene (Fig. 6).

Table 1. Infra-Red Table of the isolate (V39)

| Serial no. | Frequency cm⁻¹ | Band | Functional group |
|------------|----------------|------|-----------------|
| 1.         | 670.9          | O-H  | Alkanol         |
| 2.         | 1013.8         | C-H  | Alkenes         |
| 3.         | 1315.8         | C=Os | Alkanols        |
| 4.         | 1438.8         | C-H(vibration) | Alkanes       |
| 5.         | 1658.7         | C-C=Cmethyl | Alkanes        |
| 6.         | 2922.2         | C-H=Cmethyl | Alkanes        |
| 7.         | 3388.2         | O-H=Cmethyl | Alcohol        |

Table 2. Comparison of observed ¹H-NMR (400MHz) and ¹³C-NMR (100MHz) spectra of compound V39 in CDCl₃ with the reported values (δ) of Lupeol (¹H-NMR: 400MHz, ¹³C-NMR: 100MHz) in CDCl₃

| Position | ¹H(δ ppm) | ¹³C(δ ppm) | ¹H(δ ppm) | ¹³C(δ ppm) |
|----------|-----------|------------|-----------|------------|
| 1        | 38.84     | 38.7       | 38.84     | 38.7       |
| 2        | 28.13     | 27.4       | 28.13     | 27.4       |
| 3        | 19.17     | 3.19(Hj, dd, J=11.2, 5.0 Hz) | 79.0 | 3.2(Hm, H-3) |
| 4        | 38.74     |            | 38.9      |            |
| 5        | 55.43     |            | 55.5      |            |
| 6        | 18.45     |            | 18.3      |            |
| 7        | 34.12     |            | 34.2      |            |
| 8        | 40.97     |            | 40.9      |            |
| 9        | 50.57     |            | 50.5      |            |
| 10       | 37.31     |            | 37.2      |            |
| 11       | 21.07     |            | 21.0      |            |
| 12       | 25.27     |            | 25.2      |            |
| 13       | 38.18     |            | 38.1      |            |
| 14       | 42.97     |            | 42.9      |            |
| 15       | 27.54     |            | 27.1      |            |
| 16       | 35.72     |            | 35.5      |            |
| 17       | 45.13     |            | 45.0      |            |
| 18       | 48.13     |            | 48.3      |            |
| 19       | 48.44     |            | 48.0      |            |
| 20       | 151.14    |            | 151.0     |            |
| 21       | 29.98     |            | 29.9      |            |
| 22       | 40.15     |            | 40.0      |            |
| 23       | 28.13     | 0.83(Hh, s, Ha-23) | 28.0      | 0.85(Hh, s, Ha-22) |
| 24       | 15.52     | 0.76(Hh, s, Ha-24) | 15.5      | 0.77(Hh, s, Ha-24) |
| 25       | 16.26     | 0.76(Hh, s, Ha-25) | 16.1      | 0.78(Hh, s, Ha-25) |
| 26       | 16.11     | 0.94(Hh, s, Ha-26) | 16.0      | 0.94(Hh, s, Ha-26) |
| 27       | 14.69     | 0.95(Hh, s, Ha-27) | 14.8      | 0.97(Hh, s, Ha-27) |
| 28       | 18.15     |            | 18.0      |            |
| 29       | 109.47    | 4.70(Hh, s, H-29a) | 1.09      | 4.70(Hh, s, H-29a) |
| 30       | 19.16     | 1.65(Hh, s, H-30a) | 19.5      | 1.65(Hh, s, H-30a) |

*(Abduallah et al., 2013)

Fig 6: Proposed structure of V39: Lupeol

The triterpenoid, lupeol (3-hydroxylup-20(29)-ene), is a powerful bioactive compound present in different medicinal plants (Sturm et al., 1996; Cammereri et al., 2008). A wide range of bioactivities and bioassays of lupeol are reviewed (Gallo and Sarachine, 2009), which suggest its useful medicinal properties with a diversity of action against various ailments. This compound is reported to be antiangiogenic, antioxidative and anti-inflammatory in nature.
Isolation and Characterization of Lupeol from the Stem...  

It inhibits early responses of tumor growth induced by benzoyl peroxide (Saleem, 2008). It also plays a very important role in the normalization of lipid profile (Sudhahar et al., 2007), wound healing activity (Harish et al., 2008), protective effect in hypercholesterolemia associated with renal damage and suppression of immune factors (Bani et al., 2006).

Antimicrobial activity assay: The compound isolated from the combined n-hexane and ethyl acetate’s extracts of the stem part of Tapinanthus globiferus tagged V39 is a steroid. Steroids are among the most widely used class of drugs and their role in the therapy of pulmonary, inflammatory, dermatological and oncological diseases have been well documented (Grover et al., 2007). Isolated steroids have been reported to possess pharmacological activities such as antifungal, antibacterial and antioxidant activities (Govindappa et al., 2011).

| Test organism | Isolate (V39) | Ciprofloxacin | fluconazole | Fulcin |
|---------------|---------------|---------------|-------------|--------|
| Methicillin-resistant Staphylococcus aureus | S | R | R | R |
| Staphylococcus aureus | R | S | R | R |
| Streptococcus pyogenes | R | S | R | R |
| Streptococcus faecalis | S | S | R | R |
| Escherichia coli | S | S | R | R |
| Campylobacter jejuni | R | R | R | R |
| Helicobacter pylori | R | S | R | R |
| Pseudomonas aeruginosa | S | R | R | R |
| Vibrio cholera | R | R | R | R |
| Salmonella typhi | S | S | R | R |
| Neisseria gonorrea | R | R | R | R |
| Candida albicans | S | R | S | S |
| Candida krusei | S | R | S | R |
| Candida tropicalis | S | R | S | R |
| Aspergillus fumigatus | R | R | R | S |

Key: S ⇒ Sensitive, R ⇒ Resistance

| Test organism | Isolate (V39) | Ciprofloxacin | fluconazole | Fulcin |
|---------------|---------------|---------------|-------------|--------|
| Methicillin-resistant Staphylococcus aureus | 26 | 0 | 0 | 0 |
| Staphylococcus aureus | 0 | 35 | 0 | 0 |
| Streptococcus faecalis | 0 | 32 | 0 | 0 |
| Streptococcus pyogenes | 28 | 37 | 0 | 0 |
| Escherichia coli | 24 | 40 | 0 | 0 |
| Campylobacter jejuni | 0 | 0 | 0 | 0 |
| Helicobacter pylori | 0 | 32 | 0 | 0 |
| Pseudomonas aeruginosa | 23 | 0 | 0 | 0 |
| Vibrio cholera | 0 | 0 | 0 | 0 |
| Salmonella typhi | 27 | 40 | 0 | 0 |
| Neisseria gonorrea | 0 | 0 | 0 | 0 |
| Candida albicans | 28 | 0 | 35 | 0 |
| Candida krusei | 21 | 0 | 32 | 0 |
| Candida tropicalis | 25 | 0 | 34 | 0 |
| Aspergillus fumigatus | 0 | 0 | 0 | 37 |

Table 5 Minimum Inhibitory Concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the isolate V39 against the test microbes (μg/ml)

| Test organism                | MIC  | MBC/MFC |
|-----------------------------|------|---------|
| Methicillin-resistant S. aureus | 50  | 100     |
| S. aureus                   | -    | -       |
| S. pyogenes                 | -    | -       |
| S. faecalis                 | 25   | 50      |
| E. coli                     | 50   | 100     |
| C. jejuni                   | -    | -       |
| H. pylori                   | -    | -       |
| P. aeruginosa               | 50   | 200     |
| V. cholerae                 | -    | -       |
| S. typhi                    | 25   | 50      |
| N. gonorrea                 | -    | -       |
| C. albicans                 | 25   | 50      |
| C. krusei                   | 50   | 200     |
| C. tropicalis               | 50   | 100     |
| A. fumigatus                | -    | -       |

EMAIKWU, V; NDUKWE, IG; MOHAMMED, R; IYUN, ORA; ANYAM, JV
From the antimicrobial activity assay of the isolated compound, it can be inferred that due to the antimicrobial effects of the isolate against two Gram-positive human pathogens *Staphylococcus aureus* and *Staphylococcus pyogenes*, it may be used for the topical treatment of skin disorders like acne, pimples, and seborrheic eczema. Systemic fungal infections (fungemias) including those by *Candida albicans* have emerged as important causes of morbidity and mortality in immune-compromised patients (e.g. AIDS, cancer chemotherapy, organ or bone marrow transplantation), thus the sensitivity of the Gram-negative bacteria, *Escherichia coli* to the isolate. The sensitivity of *Escherichia coli* to the isolated compound is favorably compared to the standard drug ciprofloxacin with the reported antibacterial activity of plant metabolites that possess steroidal nuclei.

**Conclusion:** The isolated compound could be a potential source of the drug for managing *Candida krusei* and *Pseudomonas aeruginosa*. The sensitivity of the isolated compound (lupeol) against *Staphylococcus aureus* indicates that this compound can be further developed for the fight against these microorganisms and the use of the plant in the treatment of boils and skin rashes is justified since the fungi are responsible for such illness. The sensitivity of *Escherichia coli* to the isolated compound implies that the compound is a potential source of anti-fever (anti-pyretic), anti-diarrheal, anti-nausea and anti-fatigue drugs.

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