
WWJMRD 2023; 9(04): 75-83
www.wwjmrd.com
*International Journal*
*Peer Reviewed Journal*
*Refereed Journal*
*Indexed Journal*
*Impact Factor SJIF 2017: 5.182 2018: 5.51, (ISI) 2020-2021: 1.361*
*E-ISSN: 2454-6615*

**Usman Mustapha Datin**
Department of chemistry, faculty of science, Abubakar Tafawa Balewa University, Bauchi; Nigeria.

**I.Y Chindo, U.F Hassan**
Department of chemistry, faculty of science, Abubakar Tafawa Balewa University, Bauchi; Nigeria.

**Correspondence:**
**Usman Mustapha Datin**
Department of chemistry, faculty of science, Abubakar Tafawa Balewa University, Bauchi; Nigeria.

# Isolation and Identification of Bioactive Compound from The Methanolic Leaf Extract of *Eucalyptus torelliana*

**Usman Mustapha Datin, I.Y Chindo, U.F Hassan**

**Abstract**
The leaves of Eucalyptus torelliana are used in folklore medicine for the treatment of typhoid and pneumonia. In this study, Eucalyptus torelliana was investigated for the bioactive compounds present in its leaves. The plant was selected on the basis of its wide spread use in traditional herbal medicine. The leaves were collected from the plant with Bauchi metropolis in 2020. Extraction of the plant material was done with n-hexane, dichloromethane and methanol. The extracts obtained were subjected to phytochemical investigation using standard methods and revealed the presence of alkaloids, flavonoids, anthraquinones, tannins, saponins terpenoids and glycosides. The bioactivity of each extract was tested against Klebsiella pneumoniae and streptococcus pneumoniae. The bioactivity results indicated the presence of more bioactive compounds in the methanol extract compared to the dichloromethane and n-hexane extracts the probable reason might be the bioactive compounds in Eucalyptus torelliana leaves were more soluble in methanol extract (26 mm and 25 mm) compared to dichloromethane (15 mm and 5 mm) and n-hexane (no inhibition of growth). Separation and purification of compounds in the most active (methanol) extract was done using a combination of column chromatography and thin layer chromatography and this led to the isolation of one compound coded MD2 (Lupeol) with Rf value of 0.52. The compound was identified by combination of GC-MS and FTIR. The compound identified has a wide range of biological activity reported in literature review. This will justify the use of this plant in traditional medicine and indicates a promising potential for the development of medicinal agents from Eucalyptus torelliana leaves.

**Keywords:** Eucalyptus torelliana, Methanolic Leaf Extract, *Eucalyptus torelliana*, Isolation and Identification

## 1. Introduction

Research on plants with medicinal properties and identification of the chemical components responsible for their activities have proven the enduring healing potential of many plant medicines (Babu *et al*., 2018). Wild plants have always been a major source of primary healthcare and other necessities of daily life for local communities throughout the world, an indication that medicinal plants can provide the best alternative source to obtain a variety of drugs. Drugs of natural origin now play an ever more important role in medical and health care services because metabolites produced by plants constitute a major source of bioactive substances which can be used as an alternative for cheap and effective herbal drugs against common infections (Sati and Joshi, 2011).

Despite the extensive use of antibiotics and vaccination programmes, infectious diseases continue to be a leading cause of morbidity and mortality worldwide (WHO, 2017). Wide spread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics aggravates the problems. According to the World Health Report of infectious diseases of 2013, overcoming antibiotic resistance is one of the major issues of the World Health Organization for the present millennium (WHO, 2017). This scenario has been complicated by the emergence of HIV/AIDS, which renders the victims immuno-compromised and open to opportunistic infections such as chronic cough, diarrhea, candidiasis, tuberculosis, typhoid among others. At the same time, most of

the African population lives below the poverty line and cannot afford the expensive conventional medicines. These challenges call for renewed strategies on treatment, especially in the development of new antimicrobial drugs. Nigeria is well known for its rich ethno botanical wealth, particularly regarding medicinal plants (Tabuti and Mugula, 2011) which are traditionally used in the treatment of ailments and could be a good source for discovery of new, safe and biodegradable drugs (Basha et al., 2010). As wide spread as infectious diseases is in Nigeria, the number of medicinal plant species prescribed traditionally against infectious diseases runs into the hundreds (Namukobe et al., 2011). Many of these plants have a prolonged and uneventful use that may serve as an indirect testimony to their efficacy. Unfortunately, by the year 2011, only 5-15 % of the higher plants had been systematically investigated for the presence of bioactive compounds worldwide (Newman et al., 2000) and there is no report of the current status. Eucalyptus torelliana can provide an alternative source for antimicrobial drugs. However, it can only be developed and standardized if its bioactivity is known and its bioactive ingredients are identified and characterized. There is therefore need to investigate the leaf of Eucalyptus torelliana in order to understand better its chemical composition, properties, safety and efficacy.

Eucalyptus torelliana (cadaghi or cadagha) is a tree of the genus eucalyptus locally known as "Zaiti" in Hausa and mostly found in Bauchi metropolis where it is wide spread. Eucalyptus torelliana typically grows to a height of 25-30 meters (66 ft), it has a smooth, greenish to white bark, rough at the base of older trees (Chippendale and George, 2020). And it also grows tall and straight. The leaves are lance shaped or heart-shaped, flower buds in groups of three or seven, white flower and pot-like fruit, adult leaves are lance shaped to curve.

## 2. Material and Methods

Fresh leaves of Eucalyptus torelliana were collected from Bauchi Metropolis farcm3and. The identification and authentication were carried out in the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi. A voucher specimen with Herbarium number 2552 was deposited in the Herbarium.

### Preparation of extracts

The leaves were air-dried at room temperature for 21 days. The dried leaves were ground into fine powder using an electric grinder. The powdered plant materials (1 kg) were sequentially extracted three times with n-hexane, dichloromethane and methanol at room temperature for 48 hours. The extracts were filtered through cotton wool and whatman filter paper number 1 and concentrated using a rotary evaporator at 40 $^0$C to dryness. The dried extracts were transferred into sample bottles placed in a desiccator containing anhydrous sodium sulphate to remove traces of water that are present. The dried extracts were kept in tightly stoppered bottles in a refrigerator for further analysis.

## 3. Preliminary phytochemical screening of Eucalyptus torelliana leaves

Extract obtained from the concentrated fractions were subjected to various phytochemical screening test to identify the constituent of secondary metabolites using standard methods (Silver et el., 2000; Camel 2000).

### Test for Tannins (Ferric Chloride test)

Dichloromethane, n-hexane and methanol fractions (0.30 g) each was boiled with 10.00 cm$^3$ distilled water and filtered, two drops of 1.00 % ferric chloride were added to the filtrate. Formation of a green precipitate was observed in three of the extracts which indicate the presence of phenolic OH groups in the fractions (Sofowora, 1993).

### Test for Flavonoids (Shinoda test)

### Sodium Hydroxide test

The respective extracts (1.00 g) were dissolved in 2 cm$^3$ of 10.00 % sodium hydroxide solution and filtered to give yellow colour, a change in colour from yellow to colourless on addition of dilute HCl was observed only in methanol extracts which indicates the presence of flavonoids Cannel, 2002).

### Test for Anthraquinones

Extracts (1.00g) was shaken with 10.00 cm$^3$ of benzene the contents were filtered and subjected to the following test independently and respectively. 5.00 cm$^3$ of 10.00 % ammonia solution was added to the filtrate and the mixture was shaken. Pink colouration was observed in the ammoniacal layer (Lower phase) of only methanol and n-hexane that indicated the presence of free anthraquinone in the extracts and not in dichloromethane extract which indicated absence of free anthraquinone (Cannel, 2000).

### Test for Saponins (Frothing test)

Each extract (0.50 g) was agitated with water in a test tube. Frothing persisted for 15 minutes only was observed in the methanol and dichloromethane extract which indicated the presence of saponins (Cannel, 2000).

### Test for Glycoside ($FeCl_3$)

Each extract (0.50 g) was subjected to the following test independently and respectively. Concentrated sulphuric acid (5.00 cm$^3$) was added and boiled for 15 min. This was cooled and neutralized with 20% KOH. To the solution, three drops of 1 % ferric chloride solution were added and a green to black precipitate was observed in the all extracts which show the presence glycoside (Silver et al., 2000).

### Test for Terpenoids (Salkowski test)

Each plant extract (5 cm$^3$) was mixed in 2 cm$^3$ of chloroform followed by the careful addition of 3 cm3 concentrated $H_2SO_4$. A layer of the reddish brown colouration was formed at the interface thus indicated a positive result for the presence of terpenoids (Silver et al., 2000).

### Test for Steroids (Liebermann-Buchard test)

The crude plant extracts (1.00g) were separately and respectively dissolved in 10 cm$^3$ chloroform and 2 cm$^3$ drops of acetic anhydride were added followed by 10 cm3 concentrated sulphuric acid. The mixture was carefully mixed and a violet colour that changed to blue was observed in only dichloromethane and n-hexane extracts which indicated the presence of steroids and not in methanol which indicated absence of steroid (Silver et al., 2000).

**Test for Alkaloids**

The extracts (0.50 g) were separately and respectively stirred with 5.00 $cm^3$ of 1.00 % aqueous hydrochloric acid on a water bath and filtered. The filtrate (3.00 $cm^3$) was divided into three. To the first portion three drops of freshly prepared Dragendoff's reagent was added and an orange to brownish precipitate was observed only in n-hexane extract. To the second portion 1 drop of Meyer's reagent was added and white to yellowish colour precipitate was observed only in n-hexane extract. To the third portion 1 drop of Wagner's reagent was added, a reddish-brown precipitate was formed only in n-hexane extract (Cannel, 2000).

**Test for selected antimicrobial activity**

The activity of the plant extracts was tested against two bacterial strains: Klebsiella pneumoniae and Streptococcus pneumonia both of which were obtained from the Department of Medical Microbiology Abubakar Tafawa Balewa University Teaching Hospital, Bauchi. The bacterial strains were selected on the basis of the diseases against which Eucalyptus are used. The leave part of Eucalyptus species is used locally for the treatment of pneumonia and typhoid (Commonly caused by Klebsiella pneumonia and Salmonella typhi). Testing of the plant extracts for antibacterial activity was done by the modified agar disc diffusion method (Baris et al., 2008).

The most active extract will be selected for fractionation and further analysis.

**Modified Agar disc diffusion assay for bacterial screening**

The test micro-organism was aseptically inoculated on sterile Mueller Hinton agar by surface spreading to make a uniform microbial inoculum. Using sterile glass corn borers, four wells were carefully made on the agar plate without distorting the media. The same agar plate was used to test for the control drug: ciprofloxacin against the bacteria and dimethyl sulfoxide (DMSO) as a negative control. Fifty microlitres (50 /µl) of the extracet and the controls were carefully dispensed into the respective wells and the plates left on the bench for 60 minutes to allow the system stabilizes as the inoculated microorganisms adapt to the new environment, the culture plates were incubated at $37^0$C for 24 hours. Using a metric ruler, the diameter of the zone of inhibition (the diameter of the area of no growth of the micro-organism around the disc) was measured for methanol, dichloromethane and n-hexane (Baris, 2008).

**Determination of Minimum Inhibitory Concentration (MIC) using Broth dilution method**

MIC was performed on organisms that exhibited highest sensitivity, to the methanol extract, of diameter above 12 mm upon screening. Two (2.00 $cm^3$) of the test extract was serially diluted from 2-fold to 4-fold dilution in sterile Mueller Hinton broth. 0.2 $cm^3$ of the test organism was aseptically inoculated in each of the four tubes containing the extract in order of increasing dilution (500, 250, 125 and 62.5 mg/$cm^3$). Thereafter, the tubes were incubated for 24 hours (Baris et al., 2008). After incubation, the tube next to the one showing no microorganism turbidity was considered as containing the MIC of the extract in question.

**Determination of Minimum Bactericidal Concentration (MBC)**

Following the MIC determination using the Broth dilution method, after the recommended period of incubation, 4.00 $cm^3$ of solution from each of the four tubes containing the methanol extract in order of increasing of dilution (500, 250, 125 and 62.5 mg/$cm^3$) was inoculated on Mueller Hinton agar and incubated for 24 hours. The highest dilution which showed no growth of the microorganism was considered as the Minimum Bactericidal Concentration (MBC). The purpose of this test was to determine the lowest concentration in which the methanol extract will kill Strep. pneumoniae and Klebs. Pneumoniae (Baris et al., 2018).

**Isolation and Purification of active compounds from the bioactive fractions**

**Column Chromatography**

The following column conditions were employed in running the column
chromatography.

(a) Technique-gradient elution.

(b) Column - A glass column of dimensions 75 by 3.5 $cm^3$ was used.

(c) Stationary phase - Silica gel of 60 – 200 mesh size

(d) Column packing – This was done by the wet slurry method.

(e) Sample loading – The sample was loaded by the dry loading method (Cannell,
2000). The sample was dissolved in minimum amount of 100 $cm^3$ of methanol
mixed with 30.00 g of silica gel, dried, triturated and loaded on top of
the previously packed column.

Solvents System of Elution: Various solvent systems comprising 100 % hexane,
hexane/ethyl acetate mixtures (97.5: 2.5, 95: 5, 90: 10, 80: 20, 70: 30, 60: 40, 50: 50, 40; 60, 30: 70, 20: 80, and 10: 90 %) ethyl acetate 100 % and methanol 100 % were used in eluting the column.

**Thin Layer Chromatography**

The sub-fractions were subjected to thin layer chromatography using aluminum plate coated with silica gel. In the TLC, 80 % hexane: 20 % ethyl acetate were used to develop the plate and spraying with 10 % hydrosulphuric acid upon heating colour observed. The isolated compounds were subjected to spectroscopic analysis to elucidate their chemical structures (Cannell, 2000).

**Structural determination of the bioactive compounds**

The structures of the bioactive compounds were determined using spectroscopic methods (FTIR and GC-MS). The GC-MS analysis was performed on Angilent GC-MS (6890N, 5975) at A.B.U, Zaria, Kaduna state Nigeria.

**4. Results and Discussion**

**Extracts obtained from the Leaves of Eucalyptus torelliana**

**Table 1:** Percentage yield extract of E.Torelliana from 1000 g of the prepared plant leaves.

| Extract | Yield (g) | % Yield (w/w) |
|---|---|---|
| Hexane | 16.35 | 1.64 |
| Dichloromethane | 20.06 | 2.006 |
| Methanol | 97.00 | 9.70 |

The n-hexane extract yielded 16.35 g of a brown powder and showed 6 different spots from TLC, the dichloromethane extracts yielded 20.06 g of a dark green powder upon concentration with 4 different spots from TLC while methanol extract yielded 97.00 g of a dark colored powder with 2 different spots from TLC. 3.00 g of each extract was subjected to phytochemical and bioactivity tests.

**ucalyptus torelliana Leaves Extract**

**Table 2:** phytochemical screening of the leaves of Eucalyptus torelliana.

| Plytochemical extract | n-hexane extracts | Dichloromethane extracts | Methanol extract |
|---|---|---|---|
| Flavonoids | - | - | + |
| Alkaloids | + | - | - |
| Tannins | + | + | + |
| Steroids | + | - | + |
| Glycosides | + | + | + |
| Saponins | - | + | + |
| Anthraquinones | - | + | + |
| Terpenoids | + | + | + |

Key: + = Present, - = Absent

The phytochemical screening of n-hexane leaves extracts revealed the presence of alkaloids, tannins, steroids, glycosides and terpenoids while flavonoids, saponins and anthraquinones were absent. Tannins, glycosides, saponins, anthraquinones and terpenoids were present in dicloromethane while flavonoids, alkaloids and steroids were absent. Only alkaloids were absent from methanolic extract (Table 2) Tannins, terpenoids and glycosides are highly esteemed for their antimicrobial properties (Silver et al., 2011). This is in line with use of the plant for the treatment of ailments in ethnomedicine.

### 4.3 In vitro activity of the crude extracts against selected bacteria

**Table 3:** Antibacterial activity of plant extracts against Streptococcus pneumoniae and Klebsiella.

| Test Organism | Average Zone of Inhibition (mm) | | | Cipro (+) | ( – ) DMSO |
|---|---|---|---|---|---|
| | Methanol (1000mg/cm$^3$) | Dichloromethene (1000mg/cm$^3$) | n-hexane (1000mg/cm$^3$) | | |
| S. pneumoniae | 26 | 15 | 0 | 29 | 0 |
| Klebs Pneumoniae | 25 | 5 | 0 | 28 | 0 |

Legend: Antibacterial activity of the plant extract (1000 mg/cm$^3$) each in methanol, dichloromethane and n-hexane were tested against S. pneumoniae and K. pneumoniae.

A mass of 3.00 g of each extract were subjected to bioactivity test. When the activity of the plant extracts was tested against two bacterial strains namely: Streptococcus pneumoniae and Klebsiella pneumoniae respectively (Table 3), the methanol extracts zone of inhibition diameter was 26 mm and 25 mm for Streptococcus pneumoniae and Klebsiella pneumoniae respectively (Table 3); while the dichloromethane extract exhibited a zone of inhibition with a diameter of 15 mm and 5 mm against Streptococcus pneumoniae and Klebsiella pneumoniae respectively. The n-hexane extract had no inhibition of growth on any of the bacterial strains tested (Table 3). Both bacterial strains (Streptococcus pneumoniae and Klebsiella pneumoniae) were found to be susceptible to the methanol extract as the zone of inhibition diameters are 26 mm and 25 mm respectively (Table 3) and are within the range for standard antibiotics such as ampicillin (inhibition diameter of 16-27mm), doxycycline (inhibition diameter of 18-26 mm) and tetracycline (inhibition diameter 18-27 mm) as reported by the Clinical and Laboratory Standards Institute–CLSI (2007). The dichloromethane extract showed a little antibacterial activity towards Streptococcus pneumoniae and Klebsiella pneumoniae with a zone of inhibition diameter of 15 mm and 5 mm against the bacterial strains tested (Table 3). The n-hexane extract showed no antibacterial activity towards the bacterial strains tested. The bioactivity results are regarded as evidence of the presence of more bioactive compounds in the methanol extract compared to the dichloromethane and n-hexane extracts, the probable reason might be the compounds in E.torelliana leaves were more soluble in methanol compared to dichloromethane and n-hexane. Hence, this research has proved that only dichloromethane and methanol extract possessed antimicrobial properties since the methanol extract showed the largest zone of inhibition, it was considered for further tests. Its minimum inhibitory concentration (MIC) against each of the bacterial strains was investigated as well as its Minimum Bactericidal Concentration (MBC).

**Table 4:** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extract.

| Microorganism | MIC in mg/cm$^3$ | MBC in mg/cm$^3$ |
|---|---|---|
| **Methanol extract** | | |
| Streptococcus pneumoniae | 125 | 125 |

| | | |
|---|---|---|
| Klebsiella pneumoniae | 250 | Not detected |

The methanol extract showed a minimum inhibitory concentration (MIC) of 125 mg/cm$^3$ on Streptococcus pneumoniae and 250 mg/cm$^3$ on Klebsiella pneumoniae. This showed that the methanol extract is more effective against Streptococcus pneumonia compared to Klebsiella pneumoniae. The Minimum Bactericidal Concentration (MBC) of 125 mg/cm$^3$ on Streptococcus pneumoniae and none on Klebsiella pneumoniae also showed that the extract has a stronger activity towards Streptococcus pneumoniae than Klebsiella pneumoniae. The high MIC values therefore call for a higher dose for effective treatment. This result supports the high dosage (3-4 capfuls per day) used by the traditional healers for an adult (Tabuti, 2011).

### 4.4 Isolation and purification of active compounds from the fractions

**Table 5:** Fraction from column chromatography of methanol fraction.

| Fraction | Eluting solvent | Fraction number |
|---|---|---|
| 1 | Hexane (100 %) | 1-5 |
| 2 | Hexane : ethyl acetate (95: 05 %) | 6-10 |
| 3 | Hexane : ethyl acetate (90: 10 %) | 11-15 |
| 4 | Hexane : ethyl acetate (80: 20 %) | 16-20 |
| 5 | Hexane : ethyl acetate (70: 30 %) | 21-25 |
| 6 | Ethyl acetate (100 %) | 26-30 |

The methanol fraction (20 g) of Eucalyptus torelliana was chromatographed on silica gel packed column of dimension 75 by 3.5 cm$^3$ and the column was eluted continuously using 100 % n-hexane and later n-hexane: ethyl acetate mixture. 30 fractions (50 mL per fraction) were collected. The five fractions from 20 % ethyl acetate, were pooled together based on similarities on their TLC profile to give 2 sub- fractions namely MD1 and MD2. Second Sub-fraction (MD2) gave single spot as pure compound.

**Table 6:** TLC profile of the isolated compounds MD2.

| Compound code | Solvent system | Observed spots | Colour of spot on heating | Rf value |
|---|---|---|---|---|
| MD2 | Hexane: ethylacetate (4:1) | 1 | Purple | 0.53 |

TLC analysis of MD2 with hexane: ethyl acetate (4:1) gave a single homogenous spot (Rf 0.52). Spraying with 10 % $H_2SO_4$ in methanol upon heating MD2 gave purple colored spot (table 6).

### 4.5 Structural determination of the isolated compound MD2.

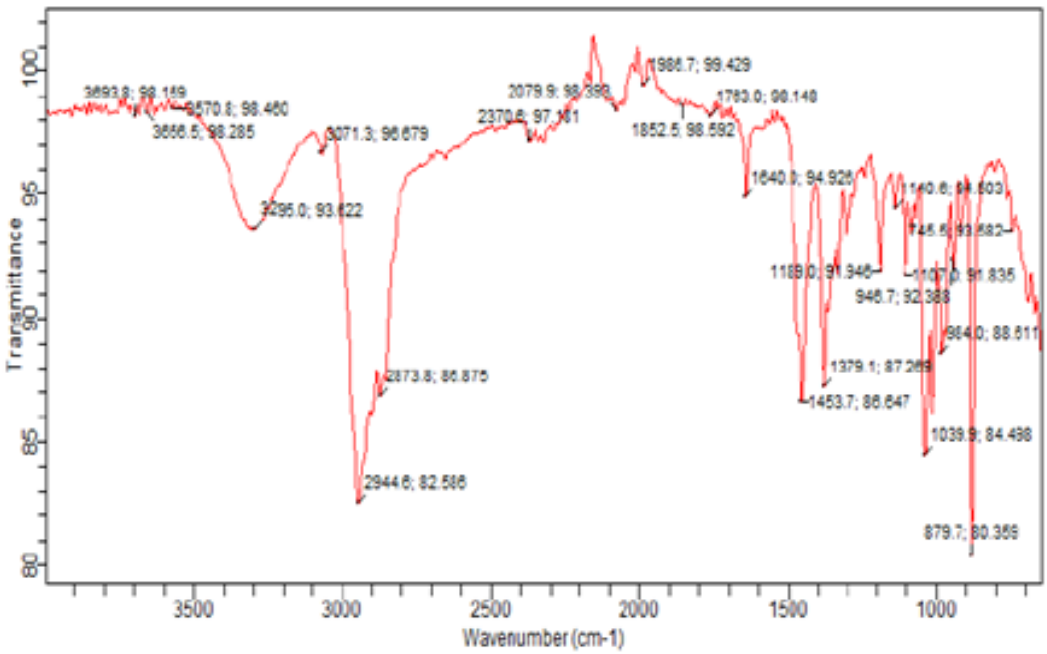



**Fig. 1:** FTIR spectrum of MD2.

Compound MD2 was isolated as white crystalline solid. The FTIR spectroscopic analysis revealed an absorption band at 3295.0 cm$^{-1}$, characteristics of phenolic O-H broad band, 2933.4 cm-$^{1}$ and 2866.3 cm$^{-1}$ for aliphatic C-H stretching. The band at1640.0 is as a result of C=C bond. 1453.7 cm$^{-1}$ a bending frequency for cyclic $(CH_2)_n$ and 1379.1 cm$^{-1}$ for $-CH_2$ $(CH)_3$. The absorption frequency at 1058 cm$^{-1}$ is typical of cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 984.0 cm$^{-1}$ (Coates, 1996).

**S**

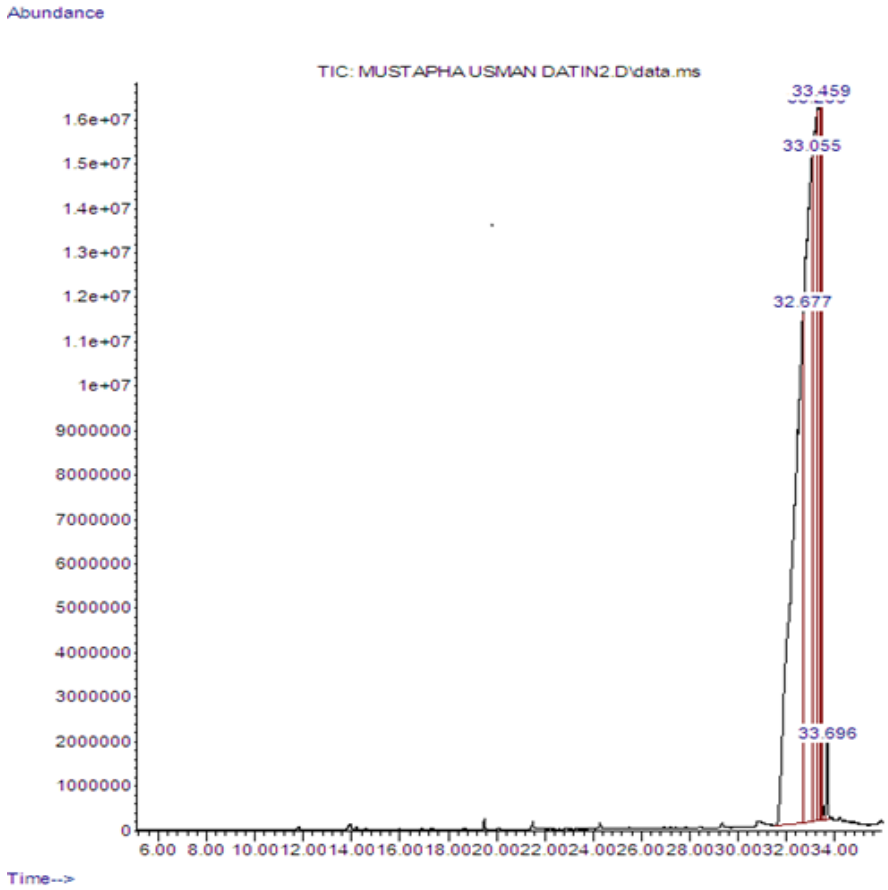
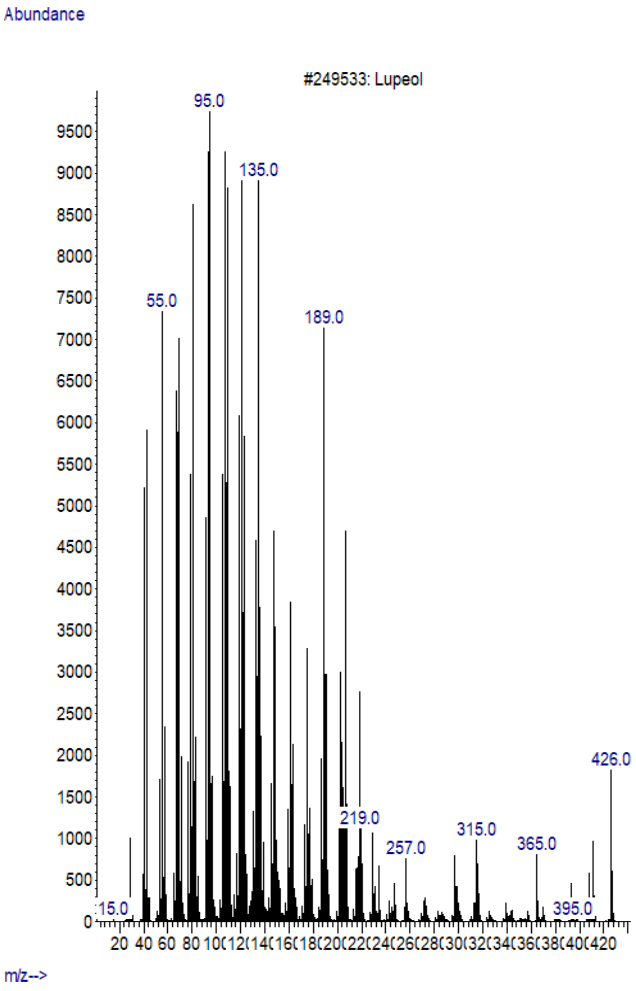



**Fig. 2:** GC-MS chromatograms of phytoconstituents MD2.



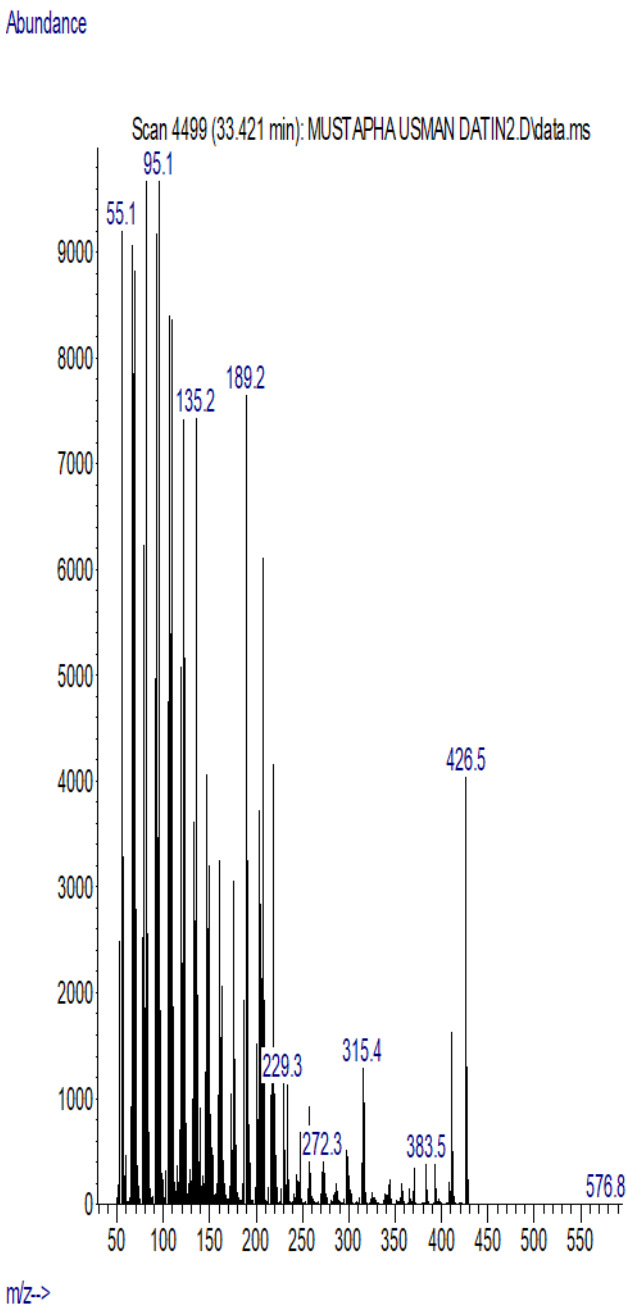



**Fig. 3:** Mass Spectra of MD2 and Library Research.

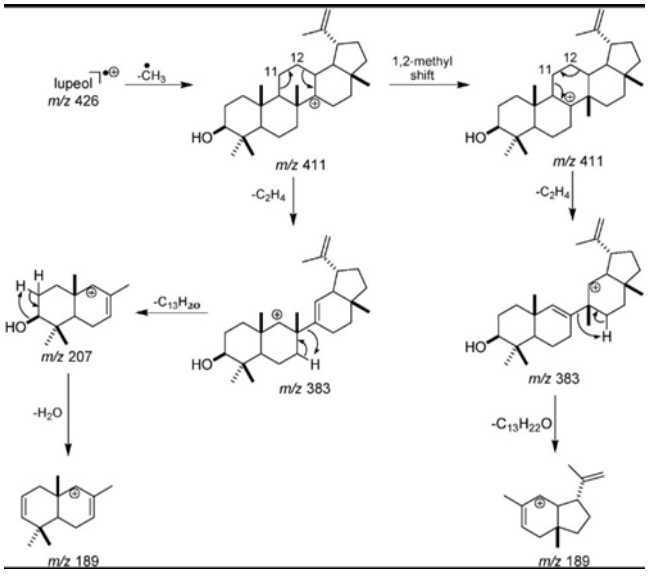
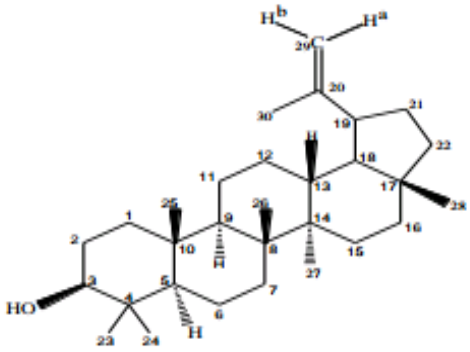



**Fig. 4:** Proposed Fragmentation pathway of Lupeol.



m/z 426

m/z 55 +

**Fig. 5:** Suggested structure of compound MD2.

GC-MS analysis the GC-MS analysis for the studied compound was performed to confirm its chemical structure. The molecular ion peak of lupeol was identified and it was found to be equal to the calculated molecular weight of the compound at m/z 426.this molecular ion underwent another cleavage to fragment methyl moiety to produce fragment of m/z 411. Researchers suggest that lupeol fragmentation starts at cleavage at C14-C27 (shown in Figure 4) followed by subsequent • CH3 removal (Noor, 2019). Fragmentation peaks at m/z 411 due to fragmentation of methyl molecule and 383 due to breaking of ethene molecule are not easily to be recognized because they readily decompose into fragment ions at m/z 189 that is suggested to be produced from two ways.

Therefore, Mass spectra of MD2 couples with the fragmentation pathway pattern one can concluded that one pure compound was found to be present in the fraction and the tentative identification was achieved by comparing the mass spectra with the data system library. The compound identified as lupeol acts as an effective antibacterial agent when tested against both gram positive and gram negative bacteria such as Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Staphylococcus lutea, Salmonella paratyphi, Salmonella typhi, Shigella boydi, Shigella dysentriae, Vibrio mimicus, E. coli, Klebsiella pneumoniae and P.aeruginosa. The reported pharmacological activities of lupeol supported the traditional use of Eucalyetus toerlliana for the treatment of cough, diarrhoea and typhoid. According Siddique and Saleem (2011), lupeol inhibited the growth of a variety of pathogenic fungal species such as Sporothrix schenckii, Microsporum canis, Aspergillus fumigates and Candida albicans; which also supported the traditional use of Eucalyptus torelliana in the treatment of candidiasis and skin diseases.

## 5.2 Conclusion

The leaves of Eucalyptus torelliana were found to contain flavonoids, tannins, glycosides, alkaloids and anthraquinones. The study also revealed the antimicrobial activities of the plant extracts and the bioactivity results were considered as evidence of the presence of more bioactive compounds in methanol extract compared to the dichloromethane and n-hexane. The compounds isolated from this plant were also isolated from different plant and their antibacterial, antitumor and anti-inflammatory activity were also reported, but this is the first report on their isolation from Eucalyptus torelliana. Therefore, the therapeutic activity coupled with the result of antimicrobial assay indicated that the leaves could be used in the treatment of the ailments acclaimed in ethno-medicines.

## 5.3 Recommendations

Based on the present research work, the followings are recommended:

1. Other parts of the plant should be screened for their antimicrobial activities so as to validate the whole plant`s antimicrobial properties.
2. Since the isolation work was made on the methanol extract only there is need to carry out work on the hexane, ethyl acetate and chloroform fractions of this plant, because they also showed some activities.
3. Structural modification of the isolated compounds for enhancement of their activities.

## References


1. Babu, N.P., Pandikumar, P. Ignacimthu, S. (2018). Anti-inflammatory Activity of Medicinal Plant, an Ethnomedicinal plant in Acute and Chronic Animal Models of Inflammation. Journal of Ethnopharmacology, 125: 356-360.
2. Baris, D. R., Singh, H. P., Kohli, R. K and Kaur, S. (2008) Eucalyptus Essential oil as a Natural Pesticide. Forest Ecol Manag 256: 2166-2174.
3. Basha, S.G., Sharief, S.D. and Shankar, S.G. (2010). Comparison of In vitro Susceptibility of Melanised and Non-melanised Cryptococcus Neoformans to Research journal of Medicinal plant, 2 (1): 34-36.
4. Bell, J., Keeling C. I (2008) Terpenoid Biomaterials. The plant journals. 54 (4): 656–669.
5. Cannell, R. J. P. (2000). Methods in Biotechnology, Natural products isolation. Human press, New Jersey. Pp 14-36.
6. Cassein, D.C., Davidson, N.J., Shields, C.B., Wiltshire, R. (2018). Reflectance and Phenolics
7. Chippendale, A.L., George M. (2020). Australian Biological Resources Study, Department of Agriculture, Water and the Environment, Canberra. Retrieved28 Febuary2020.

8. Clinical and laboratory standards institute (CLSI) (2007). Performance standards for antimicrobial susceptibility testing, seventeenth informational supplement 27 (1).
9. Coates, J.P (1996). The interpretation of infrared spectra: Publishe reference sources. Applied spectroscopic reviews, 31 (2-1) 179-192.
10. Doenhoff, MJ., Hagan, P., Southgate, V. Mbaye, A. Engels, D. (2009) Praziquantel: Its Use in Control of Schistosomiasis in Sub-Saharan African Current Research Needs. Egypt J Chem 54: 99–113.
11. Ejikeme, E. E., Lassi, Z.S., Critchley, J.A. (2011). Fluoroquinolones for Treating Typhoid and paratyphoid fever. Journal of Ethnopharmacology 136 (1): 123-128.
12. Ekwueme, F.N., Oje, O.A., Ozoemena, N.F. and Nwodo, O.F.C. (2011). Anti Inflammatory Effect of the Aqueous Leaf Extract of Sennamimosoides in Membrane Stabilization and Inhibition of Phospholipase A2 Activity. Journalof Pharmacy Research, 4 (7): 1973-1976.
13. El-Sabed, M.M., Mahmoud, M.A. and Ghareeb, M.A (2011) Chemical Constituents and Anti-oxidant Activities of the Methanolic extract of Azadirachtaindica.
14. Ghalem, B.R., Mohamed, B. (2014) Antibacterial activity of essential oil of North-West
15. Ghareeb, M.A., Refahy, L. A., Saad, A.M., Osman, N.S., Abdel-Aziz, M.S., El-Shazly, M.A., Mohamed, A.S (2015) Invitro Antimicrobial Activity of five Egyptian Plant Species. J Appl Pharm Sci. 5: 45–49.
16. Mosad, A., Ghareeb, Hanan, S.M. (2018) Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Kornish El-Nile St. 1 (2) 4-11. Murrayapaniculata. International journal of Biological Technology 1 (3), 5-9.
17. Myelogenous Leukemia (CCM3) Under in Vitro Conditions. 3: 186-190.
18. Namukobe, J., Kasenene, J.M., Kiremire, B.T., Byamukama, R., Mugisha, M.K., Krief, S., Dumonet, V., Kabasa, J.D. (2011). Traditional Plants Used for Medicinal Purposes by Local Communities Around the Northern Sector of Kibale National Park, Uganda. Journal of Ethnopharmocology 136, 236-254.
19. Newman, D.J., Cragg, G.M., Snader, K.M. (2000). The Influence of Natural Products Up on Drug Discovery. Journal of Natural Products Reaearch 17,215-234 of Green and Glaucous Leaves of Eucalyptus. Australian journal of botany. 55 (5): 561
20. Noor A. K., Widad M. K., Ibrahim S.K., Detection of Lupeol in Calendulal Officinalis Grown in Irak by G.C.M.S Analysis 2019 November7(5):357.
21. Sati, S.C. and Joshi, S. (2011). Aspects of Antifungal Potential of Ethnobotanically Known Medicinal Plants. Research Journal of Medicinal Plants 5,377-391.
22. Siddique, H. R. and Saleem, H. (2011). Beneficial health effects of lupeol triterpene: A review of preclinical studies. Life Sciences 88, 285–293.
23. Siddiqui, B.S., Begum, S., Siddiqui, S (1997) Isolation and Structural Elucidation of Acylated Pentacyclic Triterpenoids from the Leaves of Eucalyptus Camaldulensis var. obtusa. Planta Med 63: 47–50.
24. Silva, G.L., Lee, I. and Douglas, K.A. (2000). Special problems with extraction of plants.In: Cannel, J.P.R. (ed.). Natural Products Isolation. Humana press publishers, New Jersey (USA): 356-358.
25. Silver, A., Ayoub, N., Al-Sayed, E., Martiskainen, O., Sinkkonen, J and Pihlaja, K. (2011). Phenolic cconstituents of Eucalyptus Camaldulensis Dehnh, with Potential Antioxidant and Cytotoxic Activities. Rec Nat prod 5: 271-280
26. Sofowora, E. A., Introduction to Medicinal Plants and Traditional Medicine. Spectrum Books Limited. 1993; 2: 8-76.
27. Tabuti, J.R.S. and Mugula, B.B. (2011). The Ethnobotany and Ecological Status of Eucalyptus in Budondo Sub- County, Eastern Uganda. African journal of Ecology 45 (3), 126-129.
28. World Health Organisation, (WHO). (2017). Report on Infectious Diseases, Remove Obstacle to Healthy Development. World Health Organization, Geneva.