Research Square

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Chemical compositions, Antimicrobial activity and cytotoxicity of the essential oil from Mezonueron benthamianum (Baill)

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Abstract

Mezoneuron benthamianum (Baill) is a medicinal plant used traditionally in treatment of several ailments including microbial infection related diseases. The study investigate Chemical compositions, Antimicrobial activity and cytotoxicity of the essential oil from *Mezonueron benthamianum*. The essential oil from fresh leaves of *M. benthamianum* was obtained by hydro-distillation using Clevenger-type apparatus, Antimicrobial activity and cytotoxcicty of the essential oil obtained from *M. benthamianum* was evaluated using micro-dilution methods. A total of 57 compounds were identified, accounting for 84.61% of the total composition, following analysis by GC-MS. The essential oil was largely dominated by sesquiterpenes (41.08%), while a significant amount of monoterpenes were also identified. Monoterpene hydrocarbon, para-cymene (18.82%) is the most abundant constituent and the oxygenated diterpenoids were barely represented by phytone (1.03%) and phytol (1.52%). Other compounds of significant quantities are caryophyllene oxide (9.48%), beta-elemene (5.28%), carvacrol (4.81%), limonene (3.99%), pogostol (3.29%), iso-spathulenol (3.14%), trans-nerolidol (2.98%) and sesquiterpineol (2.88%). The essential oil demonstrated a remarkable activity against the Gram-positive bacteria with strongest bactericidal activity against *Bacillus subtilis* (MIC 7.81µg/mL and MBC 31.25µg/mL). Moderate inhibitory activity against *Staphylococcus aureus* with MIC and MBC values of 31.25µg/mL and 125µg/mL, respectively. *M. benthamianum* showed comparatively lower to moderate activities against Gram-negative bacteria. No inhibitory effect was observed against the fungus, *Aspergillus fumigatus* at the tested concentrations (MIC 0.00 µg/mL, MFC > 1000 µg/mL) and *Penicillium notatum* exhibited greater resistance towards *M. benthamianum* (MIC 125.00 µg/mL, MFC 500.00 µg/mL). *M. benthamianum* oil exhibited no hemolytic activity on the human red blood cells (hRBCs), in contrast to the positive control diclofenac (50 µg/mL) which showed complete hemolysis. *M. benthamianum* contain bioactive compounds with promising antibacterial and antifungi potentials which validate its traditional usage in combating microbial related infections.

Introduction

Mezoneuron benthamianum (Baill) is a genus of flowering plants in the legume family, *Fabaceae* which belongs to the subfamily *Caesalpinoideae*. The leaves are brightly red when young and dark green when old. The stem is black with reoccurred thorn. The root is brown and hard. *M. benthamianum* grows as shrubs and a woody climber of up to 8 m high and located around a deciduous secondary jungle and savannah forest from Senegal to Nigeria (Cox, 1994).

In folk medicine, the plant is for the treatment of dermal infections and wounds in Ghana. The leaves are considered in Senegal to be antiseptic and used in cleaning, healing of refractory sore. Young leaves are mashed and applied on wounds and swollen parts of the body in Sierra-Leone. The root is reported to be used as chewing stick among the yoruba southwestern Nigeria, particularly in Ibadan, where it earned the names “meyinro” and because of its thorny stem “ekanan-Ekun” (Verger, 1997).

It also used traditionally in the treatment of ulcers, dysentery, urethral discharges, skin diseases and wounds (Dickson et al, 2011). A macerate of leafy twigs is given to people suffering from impotence related to venereal diseases. The leaves are also applied externally as a paste to treat snakebites, treatment of wounds, skin infections, piles and ulcers. The stem liquid is dropped in the eye to cure inflammation and cataract (Burkill, 1985).

According to Ademoye et al (2018) and Olivia *et al* (2017), bioactive compounds in the leaves solvent extract of *M. benthamianum* include mezobenthamic acids, neocaesalpin, quercetin, kaempferol and β-sitosterol glucoside and a qualitative phytochemical screening of the leaves of *M. benthamianum* showed the presence of alkaloid, saponin, tannin, anthraquinone, flavonoids and steroids, resveratrol, gallic acid and derivatives, which exhibit antimicrobial, antiplasmodial and antioxidant activities.

Hence, *M. Benthamianum* is host to many pharmacologically potent phytoconstituents responsible for the biological activities elicited by the plant. Antiplasmodial activity of extracts and fractions were evaluated *in-vitro* against a chloroquine-sensitive strain of *Plasmodium falciparum*. Hydro-ethanolic extracts (70% v/v) of *M. benthamianum* leaves showed a moderate in vitro activity against *P. falciparum* 3D7, with IC_{50} =6.5µg/mL which is concrete evidence to support the ethnopharmacological use of *M. benthamianum* leaves extract in managing malaria (Olivia *et al*, 2017).

A study demonstrated that the ethyl acetate extract of *M. benthamianum* exhibited significant activity against oral pathogens, supporting its traditional use in oral hygiene and methanolic extract from *M. benthamianum* possessed selective cytotoxic effects against various cancer cell lines, indicating potential anticancer properties (Osamudiamen et al, 2020a, b). Also, Nwude *et al* (2023) isolated methyl gallate from methanol extract of *M. benthamianum*, which was found to be antidiabetic active through in silico study.

Synthetic antimicrobial agents like the β-Lactams, fluoroquinolones, tetracyclines, aminoglycosides e.t.c have played a central role in modern therapeutics by offering a broad-spectrum activity against pathogenic organisms. However, their clinical use is often restricted due to various degrees of adverse effects, toxicity and the emergence of drug resistant effects. Thus, biologically-active natural agents proffer an alternative solution to these side effects. The historical and traditional use of parts of *M. benthamianum* in the treatment of wounds and skin infections is a pointer that it contains some bioactive constituents that may have antimicrobial potentials. Therefore, the current study investigates the chemical compositions, antimicrobial activity and cytotoxicity of the essential oil from *M. benthamianum*.

Materials and Methods

Plant material

Fresh leaves of *M. benthamianum* were collected in March 2025 from a farm land at Egbeda village Ibadan, Oyo State Nigeria. The plant was identified and authenticated by Dr Niyi Odewo at the herbarium

of Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria, where voucher specimen (FHI113189) was deposited.

Hydrodistillation of the essential oil

The leaves of *M. benthamianum* were air dried under shade for 7–10 days, and crushed using electric blender. Aliquot (250 g) sample of air-dried and pulverized plant sample was subjected to hydro-distillation using Clevenger-type apparatus for 4 h in accordance with the British Pharmacopoeia specification (BP, 1993, Vol 1). The obtained essential oil was stored in a sealed glass bottle with a screw lid cover under refrigeration at 4 °C until ready for use.

Gas-Chromatography-Mass Spectrometry analysis of M. benthamianum essential oil

The chemical composition of the essential oil of the leaves of *M. benthamianum* was determined using gas chromatography-mass spectrometry (GC-MS) with a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans per second, and GC-MS solution software. The GC column was a ZB-5 fused silica capillary column (30m length, 0.25 mm inner diameter), with a stationary phase of 5% phenyl-polymethylsiloxane and a film thickness of 0.25 µm. Helium gas was employed as a carrier gas, with column head pressure of 552 kPa and flow rate of 1.37 mL/min. The injector and ion source temperatures were 250°C and 200°C, respectively. Initially set at 50°C, the oven temperature for the GC was progressively raised to 260°C at a rate of 2°C per minute. The sample (5% w/v) was dissolved in dichloromethane, and 0.1 µL of the solution was injected using a split-injection technique (30:1). The essential oil components were identified by comparing the retention indices calculated in relation to a homologous series of n-alkanes and the mass spectral fragmentation patterns with those recorded in the MS databases. (Adams, 2007; Mondello, L. FFNSC 3, 2016; Satyal, 2015; NIST, 2017).

Antimicrobial Assay

Bacteria and Fungi strains

The bacteria and fungi strains used were gotten from the collections of microbial stock in the Department of Biological Sciences, Covenant University (CU), Ogun State, Nigeria. The Gram-positive bacteria isolates included *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Micrococcus* species 700405. Gram-negative bacteria were *Escherichia coli* ATCC 25922, *Klebsiella oxytoca* ATCC 25983and *Pseudomonas aeruginosa* ATCC 27853. The fungal isolates are *Aspergillus fumigatus* ATCC 26934, *Penicillium notatum* ATCC 20269 and *Fusarium oxysporum* ATCC 48112.

Minimum Inhibitory Concentration (MIC) for the bacterial strains

Standard micro-tube dilution bio-assay was used to determine the MIC of the essential oil samples against the standard strains in a 96-well microtitre plate, as described by Tanhaeian et al., 2020. To each of the Wells, 20 µL of sterile 1% peptone water was added to Wells 2–10 in each row. To Well 1 and Well 2, 20 µL of the sample was placed, and serial dilution was made separately from Well 2 to Well 10 while 20 µL was discarded from Well 10. An equal volume of 20 µL of an overnight broth culture at 0.5 MacFarland turbidity of the standard strains was added to all dilution ranging from well 1 to well 10. Well 11 contains 20 µL overnight broth culture with DMSO only (negative control), and well 12 contains only 20 µL of the strain and 20 µL of streptomycin (100 ug/mL) to serve as a positive control. The plate was incubated at 37°C in ambient air for 24 hours. The minimum concentration showing no turbidity was recorded as the MIC and interpreted according to Clinical & Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018).

Minimum inhibitory Concentration (MIC) for the fungal strains

Standard micro-tube dilution bio-assay was used to determine the MIC of the oil against the standard strains in a 96-well microtitre plate as described by Khaliq et al., 2024. To each of the Wells, 20 µL of sterile 1% yeast extract was placed in Wells 2 to 10 in each row. To Well 1 and Well 2, 20 µL of the sample was placed, and serial dilution was made separately from Well 2 to Well 10 while 20 µL was discarded from Well 10. An equal 20 µL fungal spore inoculum of 0.5 MacFarland turbidity of standard strains was added to all the dilution ranging from well 1 to well 10. Well 11 contains 20 µL fungal spore inoculum with DMSO only (negative control), and well 12 contains only 20 µL of the fungal spore inoculum and 20 µL of ketoconazole (200 ug/mL) to serve as a positive control. The plate was incubated at 25°C for 3 – 5 days. The minimum concentration showing no turbidity was recorded as the MIC and interpreted according to Clinical & Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018).

Minimum Bactericidal Concentration (MBC)

The MBC is defined as the lowest dilution of the sample that kills the bacteria isolates as described by Puvača *et al*., 2024. Sub-culturing was done using two-to three-fold dilutions before the MIC was determined in each well on Nutrient Agar plates. After an overnight incubation at 37°C for 24 hours, the plates were examined for bacterial colony growth. Lack of growth indicates bactericidal activity, while growth indicates bacteriostatic activity.

Minimum fungicidal Concentration (MFC *)*

Minimum fungicidal concentration (MFC) is the lowest concentration of essential oil samples, resulting in the death of 99.9% of the fungal spore inoculum, as described by Khalid *et al*., 2024. Sub-culturing was

done using two to three-fold dilutions before each well showed the MIC onto Sabouraud Dextrose Agar plates. After incubation at 25°C for 3 – 5 days, the plates were examined for fungal spore or mycelia growth. Lack of growth indicates that the sample has fungicidal potential, while growth indicates fungistatic activity.

Cytotoxicity Assay

The toxicity of the essential oil samples was assessed based on their hemolytic activity on human red blood cells (hRBCs) using the Standard micro-tube dilution bioassay as described by de Oliveira et al., (2024) and Abdel Fattah et al., (2026). 10mL of human red blood cells were collected with permission from the blood bank at Covenant University Health center Ota Nigeria, for the purpose of this research study. A 90 µL of 10% Red Blood Cell (Hrbc) suspension was added to Wells 1 to 6. A 10 µL of the essential oil samples was added to Well 1, and serial dilutions were made from Wells 1 – 6. A 10 µL was discarded from Well 6. Well, 7 was left empty to separate the test and control wells. A positive control was set up in Well 8, in which 10 µL of diluted H_2SO_4 or diclofenac (50 µg/mL) was added to 90 µL of 10% Red Blood Cell suspension. Negative controls were set up in Well 9, containing only 10 µL of PBS with 90 µL of 10% Red blood cells. The plates were incubated at 37°C for 3 hours in an incubator with agitation every 1 hour. After incubation, the hRBCs treated with the essential oil samples were compared with Well 8 (positive control) and Well 9 (negative control).

Results and Discussion

Essential oil composition

The chemical composition of the essential oil obtained by hydrodistillation of the leaves of *M. benthamianum* was determined by GC–MS. Fifty-seven identified compounds accounted for 84.61% of the essential oil composition, with oxygenated sesquiterpenoids (31.71%) and monoterpene hydrocarbons (26.37%) being the dominant classes, while diterpenes occurred in minute amounts (2.55%)(Table 1). Caryophyllene oxide (9.48%), pogostol (3.29%), iso-spathulenol (3.14%), trans-nerolidol (2.98%), sesquiterpineol (2.88%) and β-elemene (5.28%) are the abundantly identified sesquiterpenoids, while monoterpenes present in a significant quantity include para-cymene (18.82%), limonene (3.99%) and carvacrol (4.81%). Table 1 revealed the essential oil composition of M. benthamianum, while the chromatogram of the oil is displayed by Fig. 1. The chemical constituents of the essential oil of *M. benthamianum* have not been previously reported to the best of our knowledge. However, a phytochemical study and antioxidant, antiplasmodial and anti-cancer screening of the leaf extract have been reported (Olivia Jansen et al, 2017; Ademoye et al, 2018; Oluremi et al, 2023).

Moreso, some of the identified compounds revealed in the GC-MS analysis have previously been reported to possess antimicrobial activities. Caryophyllene oxide (9.48%), spathulenol (2.29%) and nerolidol (2.98%) which occur in significant amount have been reported to possess antimicrobial and antifungal properties (Ogundajo et al., 2017; de Oliveira et al., 2015).

Other significant constituents identified such as the monoterpene hydrocarbons, limonene (3.99%) and *para*-cymene (18.82%) are known to enhance antimicrobial activity by disrupting microbial membrane integrity and increasing membrane permeability (Bassolé & Juliani, 2012). The presence of these identified bioactive chemical constituents of diverse pharmacological potentials in the oil of *M. benthamianum* might be responsible for its antimicrobial potential as evidently shown in this study.

The present study has shown that *M. benthamianum* demonstrated significant antibacterial and promising antifungi activities. The usage is also considered relatively safe and non-toxic within the tested doses. These findings validate the traditional usage of the plant in combating microbial related infections.

Antibacterial and antifungal activities

The antimicrobial activity of the essential oil of *M. benthamianum* was evaluated against six bacterial strains and three fungal strains by determining their MIC and MBC for bacteria and MFC for fungi as shown in Table 2 and Table 3 respectively. The essential oil demonstrated a remarkable activity against Gram-positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, and *Micrococcus species*. *M. benthamianum* exhibited strongest bactericidal activity against *Bacillus subtilis* among these microorganisms, given the remarkably low MIC of 7.81µg/mL and MBC of 31.25µg/mL.

Similarly, *M. benthamianum* displayed moderate inhibitory activity against *Staphylococcus aureus* with MIC and MBC values of 31.25µg/mL and 125µg/mL, respectively. The effectiveness of essential oils against this pathogen is particularly noteworthy because *S. aureus* is a major cause of food poisoning, wound infections and hospital-acquired infections (Ige et al., 2011; Anunibe *et al*., 2013; Lawani-Luwaj et al., 2024). This possibly suggests that the essential oil contains potent bioactive constituents capable of effectively interrupting the replication and metabolic processes of the pathogenic organism.

M. benthamianum showed comparatively lower activity against Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella oxytoca* while moderate activity with *Pseudomonas aeruginosa* (MIC 15.63 µg/mL, MBC 125 µg/mL). This reduced activity is understandably due to the presence of an outer membrane composed of lipopolysaccharides which acts as a permeability barrier in Gram-negative bacteria that restricts the penetration of hydrophobic antimicrobial compounds present in essential oils (Rufa'i *et al*., 2017). *M. benthamianum* oil showed no inhibitory effect at the tested concentrations against *Aspergillus fumigatus*, as indicated by MIC of 0.00 µg/mL and MFC greater than 1000 µg/mL. A moderate activity is observed against *Fusarium oxysporum* (MIC 31.25 µg/mL, MFC 125.00 µg/mL) and *Penicillium notatum* exhibited greater resistance towards *M. benthamianum* (MIC 125.00 µg/mL, MFC 500.00 µg/mL).

Cytotoxicity

The cytotoxic potential of the essential oil from *M. benthamianum* was evaluated using the hemolysis assay on human red blood cells (hRBCs). *M. benthamianum* oil exhibited no hemolytic activity (negative response) on the human red blood cells (hRBCs), in contrast to the positive control diclofenac (50

µg/mL) which showed complete hemolysis, while the negative control remained intact. The absence of hemolysis indicates that the essential oil of *M. benthamianum* is non-cytotoxic toward erythrocyte membrane at the tested concentrations. Hemolysis is a widely accepted indicator of cell membrane-disruptive toxicity, as red blood cells are highly sensitive to compounds that compromise lipid bilayer integrity. The inability of the oil to induce hemoglobin release suggests that its bioactive constituents do not significantly compromise membrane structure or induce oxidative damage to erythrocytes (Oyedemi et al., 2010; Akinpelu et al., 2021). The lack of cytotoxicity of the plant extracts is often considered an indication of a favorable safety profile and supports their possible therapeutic applications in antimicrobial and antioxidant treatments (Bakkali et al., 2008; Adebayo et al., 2020).

Finally, this result aligns with previous studies reporting that certain plant-derived essential oils exhibit selective toxicity toward microbial cells while sparing mammalian cells, likely due to differences in membrane composition (e.g., presence of cholesterol in mammalian cells) (Bakkali et al., 2008; Burt, 2004). The non-hemolytic behavior observed here validates the potential biocompatibility of the oil of *M. benthamianum*.

Conclusion

The current study revealed that the essential oil of *M. benthamianum* contain bioactive compounds with promising antibacterial and antifungi potentials. This findings validate the traditional usage of the plant in combating microbial related infections.

Declarations

Clinical trial registration Details

Not applicable. The study does not involve Clinical trial

Funding

This research received no external funding.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics, Consent to Participate, and Consent to Publish

Not applicable.

Plant guidelines

Relevant guidelines and regulations were followed while conducting the study for the use of the plant. The plant material used are wild species and was collected from a farm land with the consent of the landowner. Also, *M. benthamianum* is not an endangered or protected species in Nigeria and the plant sample was collected purely for academic research purpose, therefore, no special permit/license was required

Conflict of Interest

The authors declared that there is no conflict of interest

Author Contribution

Conceptualization: A.L.O and M.S.O;lMethodology: A.L.O, R.A.A , M.S.O, S.P, P.A and S.W;Validation and Formal analysis, R.A.A, A.L.O, S.R.B, A.O.E and A.K.W;Data curation: A.L.O and R.A.A;Writing-original draft preparation: R.A.A, A.L.O and M.S.O;Reviewing and editing: R.A.A, A.L.O, S.R.B, A.O.E and A.K.W;Supervision: A.L.O, M.S.O and S.W;Project Administration: A.L.O and M.S.O;

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

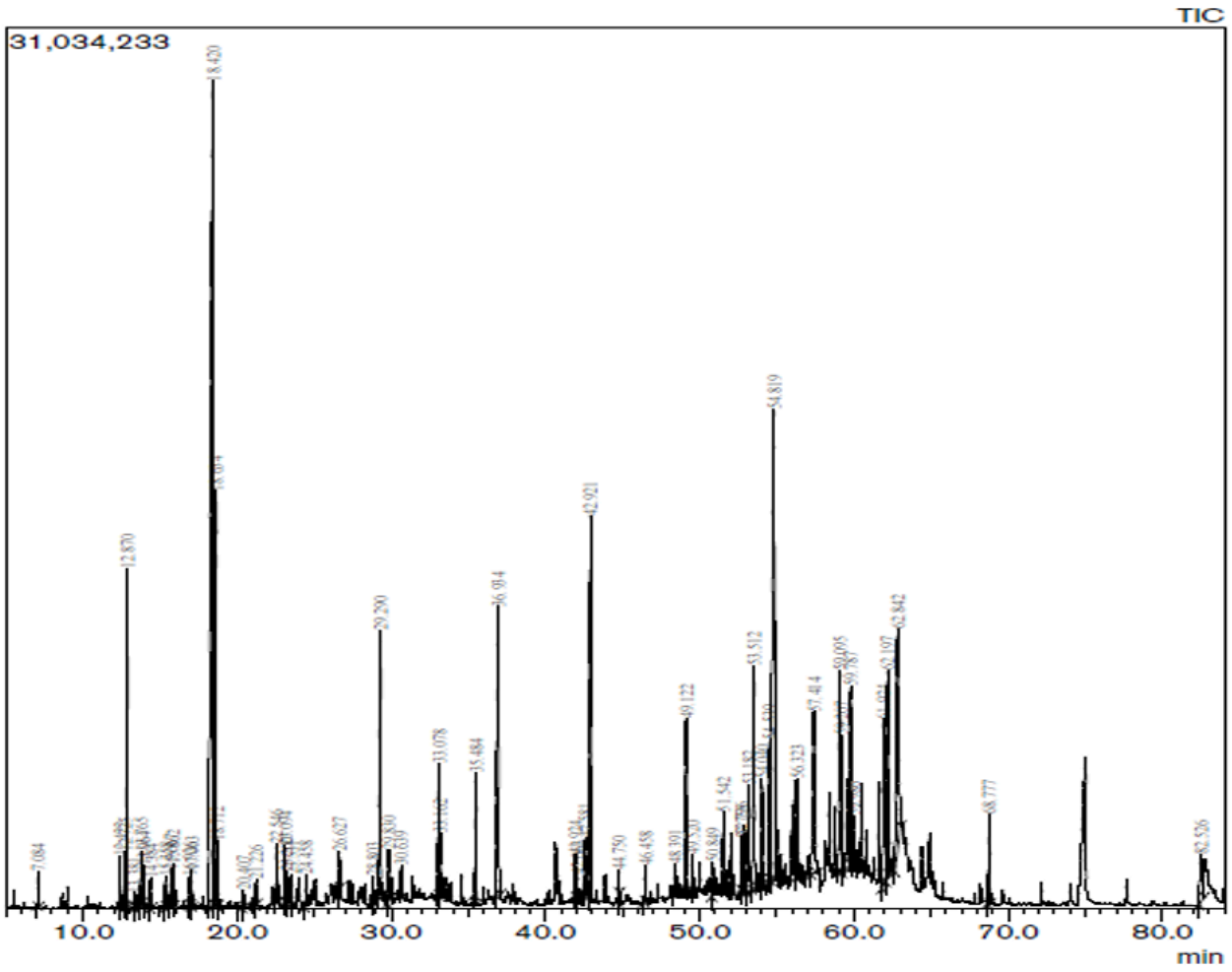


Figure 1

Chromatogram of the GC-MS analysis of the essential oil from leafs of M. benthamianum.

Supplementary Files

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