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Antibacterial Activity of a Triterpenoid from n-Butanol Extract of the Root Bark of *Ficus sycomorus* (Linn)

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ABSTRACT

Microorganisms have developed and are still developing resistance to most antibacterial agents in use. Thus, there is a need to search for new drugs that are of organic origin from medicinal plants, because they serve as a primary source of new medicine and lead compounds for the development of new antibacterial agents. The aim of this study was to isolate, characterise and investigate the antibacterial activity of lupeol from the root bark of *Ficus sycomorus.* Phytochemical investigation of n-butanol extract from the root bark of *Ficus* sycomorus showed the occurrence of steroids, triterpenes, flavonoids, alkaloids, and tannins following microwave-assisted extraction. A triterpenoid compound, lupeol, was isolated from the root bark of the plant. The isolated compound was screened for antibacterial activity against *Escherichia coli*, *Samonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* using agar well diffusion method. The isolated compound exhibited antibacterial activity against some of the tested microorganisms; *B. subtilis* and *E.* coli. This showed that the compound may serve as a potential source of antibacterial agent.

Keywords: *Ficus sycomorus,* lupeol, Microwave-Assisted Extraction, root bark.

Introduction

Ficus sycomorus (Linn) from the Mulberry family, is a large, spreading tree up to about 30 m tall with wide branches and a thick trunk extending to 3.5 m in diameter.[1] In Nigeria, it is commonly known by the Hausas as "Farin Baure", as "Ji - ewu" in Igbo language and among the Yoruba people as "Epin".[2 - 4]

The microwave - assisted extraction (MAE), is a method of extraction of phytochemicals from the plant material that works more favourably with polar solvent and it offers some advantages, over conventional extraction methods, such as faster extraction rates, purity of crude extracts, purity and higher recovery of isolates, improved stability, reduced processing costs, reduced energy and solvent usage.[5, 6] Various solvents have been used to extract different phyto - constituents and alcohol among other solvents, in any case, is a good all-purpose solvent for preliminary extraction.[7] However, El - Sayed and his co-workers reported that n - butanol (BuOH) fraction of *Ficus sycomorus* leaves has strong antioxidant activity and the compounds isolated from it were found as major components and principally responsible for the antioxidant activity of *F.* sycomorus.[8]

Several bioactive chemical constituents such as alkaloids, tannins, flavonoids and phenolic compounds have been reported in *Ficus sycomorus*,[9, 10] and the root bark of the plant is an important medicine used traditionally for the treatment of epilepsy, diarrhoea, dysentery, urinary tract, vaginal infections and phathologic hemorrhoid.[4, 11] However, to the best of our knowledge there is no study on microwave - assisted extraction (MAE) and the study on the phytochemicals characterization from the root bark of this plant is scanty.

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This paper reported the microwave - assisted extraction (MAE), isolation, characterization and antibacterial investigation of lupeol from the root bark of *Ficus sycomorus*.

Materials and Methods

General experimental procedure

Silica gel (60 - 20 mesh) was used for column chromatography, whereas Thin - Layer chromatography (TLC) and Preparative TLC were performed on aluminium plates coated with silica gel 60 F_{254}. The spots were visualized under ultraviolet light (254 and 366 nm) and by spraying with 10% H_2SO_4, followed by heating for 10 - 15 minute. The IR spectrum was recorded on a Shimadzu FTIR 400 FTIR Spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 600 MHz Bruker AVANCE spectrometer and Suart automatic melting point/SMP40 was used for the melting point determination.

Plant material

The roots of *Ficus sycomorus* was collected around Tsauni Basawa, Samaru - Zaria, in Kaduna State, Nigeria in March, 2017. The plant was identified in the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and was subsequently assigned a voucher specimen number, 1466. The root bark of the plant was removed, air – dried and pulverized. The pulverized plant material was stored in an air - tight polythene bag until ready for analysis.

Microwave - Assisted Extraction (MAE)

The pulverized plant material (600 g) was divided into five different portions, soaked in n-butanol and allowed to stand overnight. Afterward, they were placed in a microwave oven (MATSUI M180TC) under the lowest power and microwaved 5 times at 3 minutes pulses, removed and allowed to cool in between pulses. The microwaved plant material was then washed exhaustively with the same solvent. The extract was concentrated by allowing the solvent to evaporate at room temperature to afford a gummy dark red product (22.0 g).

Preliminary Phytochemical Screening
Preliminary phytochemical screening for secondary metabolites was carried out on the crude n - butanol extract of the root bark of *Ficus sycomarus* using standard methods.[12 - 14]

Isolation and purification
The butanol extract (BE) was dissolved in chloroform - ethanol (4:1) and the decant so obtained was dried at room temperature. The dried decant (10 g) was fractionated by open column chromatography with silica gel (40 g). The elution was started with 100% n-hexane and 5% increment of polarity using ethyl acetate was applied after 200 mL collection in 10 mL per fraction. Fractions 8 - 10 were combined and further separated by column chromatography on silica gel (20 g) and an isocratic elution was maintained at 95% n-hexane and 5% ethyl acetate collected at 5 mL per fraction. Compound MB01 (30 mg) was isolated from combined fractions 2 – 6 after preparative TLC developed with 98% n-hexane and 2% ethyl acetate.

Determination of Antibacterial Activity
Clinical isolates of bacteria obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria were used as test organisms for this analysis. The isolates were *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus*. Antibacterial activity was measured using agar well diffusion method, and all the media were prepared and sterilized by autoclaving at 121ºC for 15 minutes in an Astral Scientific autoclave according to the National Committee for Clinical Laboratory Standard (NCCLS).[15] The inoculum of the test organisms were prepared by first streaking the organisms on the prepared nutrient agar plates to obtain discrete colonies of the bacteria. A colony was picked and sub-cultured unto sterile nutrient broth and incubated at 37ºC for 24 hours. A loopful of each bacterial suspension, from the broth culture, was transferred into bottles containing sterile distilled water to obtain a bacterial density of 1.5×10^8 cfu/mL as determined by the McFarland scale No 1. [16]
Inhibitory activity (sensitivity test) of the extract and the isolated compound was determined by sterilizing the standardized inocula of the bacterial isolates on Mueller Hinton Agar plates; four wells on the plate were properly labeled according to different concentrations of the extract and the isolated compound, prepared by serial dilution which were 100, 50, 25, 12.5, 6.25, 3.125 mg/mL and µg/mL of the extract and the isolated compound, respectively. Each of the well was filled with approximately 0.2 mL of the different concentration of the extract or the isolated compound. The inoculated plates with the extract or the isolated compound were allowed to stay on a bench for an hour to enable the sample to diffuse into the agar. The plates were then incubated at 37ºC for 24 hours. At the end of the incubation period, the plates were observed for any evidence of inhibition which appeared as clear zone that was completely devoid of growth around the wells. The diameters of the zones were measured using a transparent and calibrated ruler.
The minimum inhibitory concentration (MIC) of the extract and the isolated compound were determined using tube dilution method with the Mueller Hinton Broth used as a diluent. The lowest inhibitory concentration from the sensitivity test was serially diluted and added into the test tubes containing Mueller Hinton Broth and the organisms, the tubes were then inoculated. The inoculated tubes were incubated at 37ºC for 24 hours. At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the Minimum Inhibitory Concentration (MIC).
Minimum Bactericidal Concentration (MBC) of the extract or the isolated compound was determined from the result of the Minimum Inhibitory Concentration (MIC). A sterilized wire loop was dropped into the test tubes that did not show turbidity (clear) in the MIC test and the loopful was taken and streaked on a sterile nutrient agar plate. The plates were incubated at 37ºC for 24 hours. At the end of the incubation period, the plates were observed for the presence or absence of growth. Growth indicated bacteriostatic activity while no growth indicated bacteriocidal activity of the extract or the isolated compounds.

Results and Discussion

Microwave - Assisted Extraction (MAE) and phytochemical analysis
The pulverized plant material of the root bark of *Ficus sycomorus* (600 g) was extracted using microwave - assisted extraction with 100% n-butanol and the percentage recovery was calculated (3.66%). The results of the phytochemical analysis of the root bark extract of *Ficus sycomorus* revealed the presence of alkaloids, flavonoids, steroids/triterpenes and tannins, whereas saponins were absent in the extract.

Isolation and characterization
Physical and chemical properties: The compound MB01 was isolated as a white crystalline solid (30 mg) with melting point of 213 - 215ºC. The retention factor (R_f) of 0.36 was recorded on TLC using n - hexane: ethyl acetate (9:1) as the solvent system. *Chemical test on the isolated compound:* The isolated compound (MB01) showed a positive test to the Liebermann - Buchard test and a negative result was observed for the Ferric Chloride test (Table 1), thus confirming that the compound is a steroid/triterpenoid.

Spectroscopic analysis: The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum showed seven methyl signals at δH 1.53, 1.04, 0.98, 0.94, 0.83, 0.78 and 0.76 ppm. A doublet of doublets at δH 3.21 ppm for H - 3 (1H, *dd*, *J* = 5.14, 11.10 Hz, H - 3). Doublets for geminal protons at δH 4.69 and 4.57 ppm for C-29 (2H, *dd*, *J* = 1.78, 8.58 Hz, H - 29a, 29b), along with the methyl signal at δH 1.53 ppm for C-30, suggested that compound MB01 was a lupine-type triterpenoid. The ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum further suggested that the MB01 was a lupine-type triterpene derivative; a total of 30 carbon signals were observed. The characteristic pair of sp2 hybridized carbon atoms was observed at δ 151.14 and 109.47 ppm. Oxygenated carbon signal was observed at δ 79.15 ppm. Consequently, after comparing the NMR data with data in the literature,[17] compound MB01 was assigned to be (3*β*)-Lup-20(29)-en-3-ol, more commonly known as lupeol ($C_{30}H_{50}O$).
The suggested characteristic signals in the ^{1}H- and ^{13}C-NMR assignment was complemented by the FTIR spectrum; a very intense broad band at 3421 cm^{-1} and moderately intense band at 1192 cm^{-1} indicated the characteristic hydroxyl group (-OH). The corresponding C=C vibrations was shown around 1662 cm^{-1}, a weakly intense band. The stretching vibration of methyl and methylene were noticed by the intense bands at 2855 and 2929 cm^{-1}, respectively. The IR absorbance values were in concordance with Silverstein *et al*.[18]

Antibacterial Activity
The n-butanol extract (BE) and the isolated compound (MB01) were screened *in vitro* for their antibacterial activity against two Gram negative (*Escherichia coli* and *Samonella typhi*) and two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria. The extract and the isolated compound showed Antibacterial activity against the tested organisms with zones of inhibition ranging from 12 - 15 mm for n-butanol extract (BE) on *B. subtilis* only with MIC and MBC values of 12.5 mg/mL and 25 mg/mL, respectively, while the isolated compound (MB01) showed activity against *B. subtilis* and *E. coli* with zones of inhibition ranging from 14 - 18 mm and 12 - 16 mm, respectively, with the corresponding minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 12.5 µg/mL and 25 µg/mL, respectively (Tables 2 - 4).
The presence of lupeol (MB01) could be linked to the observed antibacterial properties in the root bark of the plant, because lupeol have been reported to have antioxidants, immunomodulatory, antimicrobial, anti-inflammatory, anticancer and antitubercular activities.[19-22] The compound (MB01) had higher antibacterial activity compared to the crude n-butanol extract (BE). This could be due to the presence of a mixture of numerous chemical compounds with different functional groups and properties in the extract which could exhibit antagonistic interactions among themselves, or the active metabolites would be in small concentrations to exert activity against the tested microorganisms at the tested concentrations. However, the zones of inhibition (12 - 18 mm) of the isolated compound against the tested organisms was much lower compared to the standard drug (ciprofloxacin) with zones of inhibition of 26 - 27 mm.
The sensitivity of *B. subtilis* and *E. coli* to the isolated compound implies that the compound may be a potential source of antibacterial agent that could be used for the treatment of bacterial infections.[23, 24]

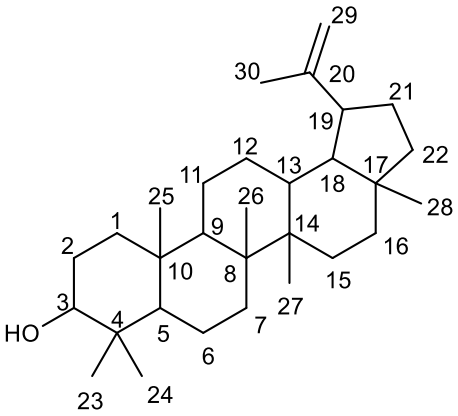

Figure 1: Chemical structure of compound MB01.

Table 1: Chemical test on the isolated compound.

Isolate	Test	Result	Inference
MB01	Ferric Chloride test	–	Non - phenolic
	Liebermann test	+	Terpenoid/steroid

Key: + = positive result, – = negative result

Conclusion

The results of the phytochemical analysis of the extracts of the root bark of *Ficus sycomorus* revealed the presence of alkaloids, flavonoids, steroids/triterpenes and tannins. The study reported the isolation of a lupane-type triterpenoid; lupeol from the root bark of *Ficus sycomorus*. The sensitivity of the tested bacteria to the isolated compound and MIC values suggests that the compound may be a potential source of antibacterial agent.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 2: Zones of Inhibition (mm) of the extracts, isolated compound and standard drug.

Extract/compound		zone of inhibition (mm)			
	Conc	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
BE (mg/mL)	100	0	15	0	0
	50	0	14	0	0
	25	0	12	0	0
	12.5	0	0	0	0
MB01 (µg/mL)	100	0	18	16	0
	50	0	16	14	0
	25	0	14	12	0
	12.5	0	0	0	0
Ciprofloxacin (µg/mL)	30	31	26	27	28

BE = n-butanol extract, MB01= Lupeol

Table 3: Minimum inhibitory concentration (MIC).

Extract/compound	Microorganism			
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*
BE (mg/mL)	ND	12.5	ND	ND
MB01(µg/mL)	ND	12.5	12.5	ND

BE = n-butanol extract, MB01= Lupeol, ND = not determined, (#) = inhibition observed.

Table 4: Minimum bactericidal concentration.

Extract/compound	Microorganism			
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*
BE (mg/mL)	ND	25	ND	ND
MB01(µg/mL)	ND	25	25	ND

BE = n-butanol extract, MB01= Lupeol, (#) = bacteriocidal, ND = not determined.

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