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Contributed Paper

Bioactive Compounds from *Mesua ferrea* Stems

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ABSTRACT

The chemical constituents and biological activities of *Mesua ferrea* Linn. were studied. The stems of *M. ferrea* were extracted with hexane, dichloromethane and methanol, respectively. The methanol extract exhibited the highest antibacterial activity against *Escherichia coli* with the MIC value of 31.25 μg/mL. The methanol extract and the dichloromethane extract exhibited the highest activity against *Staphylococcus aureus* with the MIC values of 31.25 μg/mL. The dichloromethane extract exhibited anticancer activities against KB-oral, MCF-7 and NCI-H187 cell lines with the IC_{50} values of 18.01, 28.83 and 18.42 μg/mL, respectively. Friedelin, the mixture of α-amyrin and β-amyrin, lupeol and β-sitosterol were isolated from the active dichloromethane extract of the stems. Friedelin, the mixture of α-amyrin and β-amyrin, lupeol and β-sitosterol showed the antibacterial activity against *E. coli* with the MIC values of 250, 250, 250 and 1000 μg/mL, respectively. They also showed antibacterial activity against *S. aureus* with the MIC values of 250, 250, 500 and 1000 μg/mL, respectively. The mixture of α-amyrin and β-amyrin exhibited anticancer activity against MCF-7 cell line with the IC_{50} value 28.45 μg/mL and lupeol exhibited anticancer activities against KB, MCF-7 and NCI-H187 cell lines with the IC_{50} values of 30.12, 34.25 and 21.56 μg/mL, respectively. All the extracts and the isolated compounds were non-cytotoxic to *Vero* cells.

Keywords: *Mesua ferrea*, friedelin, α-amyrin, β-amyrin, β-sitosterol, lupeol, antibacterial activity, anticancer activity

1. INTRODUCTION

Mesua ferrea L. (family Guttiferae) commonly known as Nagakeshara widely distributed in tropical countries like india, Burma, Thailand, Indochaina and New guinea [1]. It has been used as antiseptic, anti-inflammatory, hepatoprotective, diuretic, blood purifier, anthelmintic, cardiotonic, purgative, expectorant, antioxidant, antipyretic, antineoplastic, antiallergic, antiasthmatic, central nervous system (CNS), antimicrobial, depressant, antispasmodic, antivenom analgesic, and immunostimulant activities. The bark is used for treatment of cough, dysentery, vomiting, sore throat and fever. The flowers are astringent and stomachic. The leaves and flowers are used for the

treatment of snake bite and scorpion sting. The seed oil is used as an embrocation in rheumatism and used for treatment of itch [2-3]. The leaf oil exhibited antioxidant, antibacterial and anticancer activities [4]. The chemical constituents of *M. ferrea* have been reported to contain coumarins, xanthones, terpenoids and steroids. Mesuol, mammeigin and mammeisin were isolated from the seed oil [5-6]. Mesuol and mesuone showed antibacterial activity against *S.aureus* and *Mycobacterium phlei* [7]. Mesuol also showed immunomodulatory activity [8]. The heartwood of *M. ferrea* was found to contain 1, 5-dihydroxyxanthone, euxanthone 7-methyl ether, β-sitosterol, 1, 5-dihydroxy-3-methoxy xanthone and 1, 5, 6-trihydroxyxanthone [9-10]. Ferruol A was isolated from the trunk bark [11]. The stamens contained α- and β-amyrin, β-sitosterol, mesuaferrones A and B, mesuanic acid, 1, 5-dihydroxyxanthone and euxanthone-7-methyl ether [3]. Friedelin and stigmasterol were isolated from the stem bark [12]. 12,13-furano-8-hydroxy napthyl-6-0-b-2′,3′,4′,6′ tetrahydroxy-5′, 5′ dimethyl cyclohexyl ether was isolated from the leaves [13]. Mesuaferrin A, mesuaferrin B, caloxanthone C, 1, 8-dihydro-3-methoxy-6-methylantraquinone, β-sitosterol, friedelin and botulinic acid were isolated from the root bark [14]. 4-Alkyl- and 4-phenyl coumarins were isolated from the blossoms that showed antiprotozoal agents and antibacterial activity [15].

The aim of this study was to investigate the chemical constituents from *M. ferrea* stems and its antibacterial and anticancer activities.

2. MATERIALS AND METHODS

2.1 Plant Material

The stems of *Mesua ferrea* Linn. were collected from Payap University, Chiang Mai, Thailand in December 2010. The specimen was identified by J. F. Maxwell, a botanist at the Herbarium of Biology Department, Faculty of Science, Chiang Mai University where a voucher specimen has been deposited under the code number Sukanya 02.

2.2 Gerneral Experimental Procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX 400 MHz NMR spectrometers (Karlsruhe, Germany) using tetramethylsileane (TMS) as the internal standard and $CDCl_3$ as the solvents. The chemical shifts were reported in parts per million (ppm). The melting points were determined with a Gallenkamp apparatus and record with correct. Infrared spectra were recorded on a FT-IR spectrometer (Tensor 27, Bruker Optics). Spectra of the isolated compounds were recorded as potassium bromide (KBr) pellets. The Gas Chromatography-Mass Spectrometry (GC/MS) analysis was performed on the Hewlett-Packard GC6850 coupled with a HP 5973N mass selective detector. Column Chromatography (CC) was carried out using silica gel (Merck) and thin layer chromatography (TLC) was carried out on aluminium-baked Merck silica gel 60 F254.

2.3 Extraction

The stems of *M. ferrea* were dried in a hot air oven at 40°C for 26 h. and then ground into powder. The stems powder (1.82 kg) was extracted sequentially with 4 L of hexane, dichloromethane and methanol at room temperature for 7 days. All extracts were evaporated to dryness under reduced pressure using a rotatory evaporator to give the crude extracts.

2.4 Isolation and Purification

The dichloromethane extract of *M. ferrea* stems (19.11 g) was separated by column

chromatography (CC) on silica gel and using gradient solvent system of EtOAc/hexane and MeOH/EtOAc to give 6 fractions (MF1 -MF6). Fraction MF3 (3.30 g) was separated by CC on silica gel and eluted with 0-20% EtOAc/hexane to give 7 fractions (MF3.1-MF3.7). Fraction 3.2 (868.4 mg) was further separated by preparative thin layer chromatography (PTLC) and eluted with 5% EtOAc/hexane to give 3 fractions (MF3.2A1-MF3.2A3). Fraction MF3.2A2 (39.0 mg) was separated by CC on silica gel and eluted with 5% EtOAc/hexane to afford compound **1** (10.1 mg). Fraction 3.4 (240.9 mg) was separated by CC on silica gel and eluted with 10% EtOAc/hexane to give 3 fractions (MF3.4A1-MF3.4A3) and fraction MF3.4A2 (109.8 mg) was separated by PTLC and eluted with 70% CH_2Cl_2/ hexane to afford compound **2** (15.4 mg). Fraction 3.6 (128.5 mg) was separated by CC on silica gel and eluted with 20% EtOAc/ hexane to afford compound **3** (36.2 mg). Fraction MF3.4 (7.04 g) was separated by CC on silica gel and eluted with gradient solvent system of EtOAc/hexane to give 6 fractions (MF4.1-MF4.6). Fraction 4.3 (60 mg) was further separated by PTLC and eluted with 20% EtOAc/hexane to afford compound 4 (16.6 mg).

Friedelin (**1**) : white powder; mp. 261.5-263.0°C (lit. 262.0-263.0°C [16]); IR (KBr) ν_{max} 2,924, 2852, 1739 and 1463 cm^{-1} ; MS *m/z* (%) 426(M^+,24), 411(12), 341(10), 302(40), 273(73), 205(40), 123(70), 109(85), 95(80), 81(68) and 69(100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.72 (3H, *s*, Me-24), 0.87 (3H, *s*, Me-25), 0.87 (3H, *d*, *J* = 6.82 Hz, Me-23), 0.95 (3H, *s*, Me-29), 0.95 (1H, *m*, H-22a), 1.00 (3H, *s*, Me-26), 0.99 (3H, *s*, Me-30), 1.05 (3H, *s*, Me-27), 1.17 (3H, *s*, Me-28), 1.25 (1H, *m*, H-6a), 1.33 (1H, *m*, H-7a), 1.39 (1H, *m*, H-8), 1.40 (1H, *m*, H-7b), 1.48 (1H, *m*, H-22b), 1.55 (2H, *m*, H-10), 1.57 (1H, *m*, H-18), 1.70 (1H, *m*, H-1a), 1.74 (1H, *m*, H-6b), 1.96 (1H, *m*, H-1b), 2.24 (1H, *m*, H-2a), 2.25 (1H, *m*, H-4), 2.37 (1H, *m*, H-2b); ^{13}C-NMR ($CDCl_3$, 100 MHz): d 22.3 (C-1), 41.5 (C-2), 58.3 (C-4), 42.1 (C-5), 41.3 (C-6), 18.2 (C-7), 53.1 (C-8), 37.5 (C-9), 59.5 (C-10), 35.6 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.5 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.4 (C-19), 28.2 (C-20), 32.6 (C-21), 39.3 (C-22), 6.8 (C-23), 14.7 (C-24), 18.0 (C-25), 20.3 (C-26), 18.7 (C-27), 32.1 (C-28), 35.0 (C-29) and 31.8 (C-30).

The mixture of *α*-amyrin and *β*-amyrin (**2**) : white powder; mp. 188.0-193.0°C; IR (KBr) 3373, 1651-1601, 1427 and 1097 cm^{-1} ; MS of peak at t_R = 80.53, *m/z* (%): 426(M^+,5), 218(100), 203(45), 189(13), 175(5) and 109(6); MS of peak at t_R = 82.11, *m/z* (%): 426(M^+,8), 218(100), 203(15), 189(7), 175(3) and 109(7); ^{1}H-NMR of ($CDCl_3$, 400 MHz); *α*-amyrin : δ 0.74 (3H, s, Me-25), 0.90 (3H, s, Me-26),0.95 (3H, s, Me-28), 0.86(3H, *d*, *J* = 6.21 Hz, Me-29), 0.72 (3H, *d*, *J* = 6.84 Hz, Me-30), 0.78 (3H, s, Me-24), 0.93 (3H, s, Me-23), 1.03 (3H, s, Me-27), 3.21(1H, *dd*, *J* = 5.05, 11.12 Hz, H-3), 5.12 (1H, *t*, *J* = 3.54 Hz,H-12); *β*-amyrin : δ 0.74 (3H,*s*, Me-25), 0.78 (3H, *s*, Me-23), 0.82 (3H, *s*, Me-30), 0.89 (3H, *s*, Me-29), 0.92 (3H, *s*, Me-24), 0.95 (3H, *s*, Me-26), 1.06 (3H, *s*, Me-28), 1.22 (3H, *s*, Me-27), 3.20 (1H, *dd*, *J* = 10.86, 4.55 Hz, H-3) and 5.16 (1H, *t*, *J* = 3.54 Hz, H-12).

β-Sitosterol (**3**) : white powder; mp. 138.0-140.0°C (lit. 138-139°C [17]); IR (KBr) 3253, 2936, 2850, 1662, 1462, 1381 and 1049 cm^{-1} ; MS *m/z* (%) 414(M^+,100), 396(85), 381(45), 329(64), 303(65) and 213(70); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.68 (3H, *s*, H-18), 0.82 (3H, *d*, *J* = 7.07 Hz, H-27), 0.83 (3H, *d*, *J*= 7.32 Hz, H-26), 0.86 (3H, *t*, *J*= 7.58 Hz, H-29), 0.93 (3H, *d*, *J* = 6.57 Hz, H-21), 1.01 (3H, *s*, H-19), 3.52 (1H, *m*, H-3),

5.35 (1H, *d*, *J* = 5.24 Hz, H-6); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 37.25 (C-1), 31.65 (C-2), 71.89 (C-3), 42.18 (C-4), 140.75 (C-5), 121.70 (C-6), 31.89 (C-7), 31.81 (C-8), 51.10 (C-9), 36.45 (C-10), 21.10 (C-11), 39.72 (C-12), 42.40 (C-13), 56.76 (C-14), 24.27 (C-15), 28.21 (C-16), 56.05 (C-17), 11.84 (C-18), 19.40 (C-19), 36.13 (C-20), 18.77 (C-21), 33.81 (C-22), 25.95 (C-23), 45.82 (C-24), 29.19 (C-25), 19.80 (C-26), 19.08 (C-27), 23.10 (C-28) and 11.96 C-29).

Lupeol (**4**) : white powder; mp. 213.0-214.5°C (lit. 213°C [18]); IR (KBr) 3314, 2944, 2852 and 1454 cm^{-1} ; MS *m/z* (%) 426(M^+,25), 411(5), 315(5), 257(6), 218(40), 207(62), 189(51), 109(64), 68(98) and 43(100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.68 (1H, *m*, H-5), 0.76 (3H, *s*, H-24), 0.79 (3H, *s*, H-28), 0.83 (3H, *s*, H-25), 0.93 (3H, *s*, H-27), 0.96 (3H, *s*, H-23), 1.05 (3H, *s*, H-26), 1.68 (3H, *s*, H-30), 1.26 (1H, *m*, H-21), 2.37 (1H, *dt*, *J* = 11.10, 5.70 Hz, H-19), 3.18 (1H, *dd*, *J* = 11.12, 5.24 Hz, H-3), 4.55 (1H, *brs*, H_b-29), 4.67 (1H, *brs*, H_a-29); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 38.6 (C-1), 27.4 (C-2), 79.2 (C-3), 39.7 (C-4), 55.4 (C-5), 18.7 (C-6), 34.2 (C-7), 40.9 (C-8), 50.5 (C-9), 37.2 (C-10), 20.9 (C-11), 25.3 (C-12), 38.2 (C-13), 42.8 (C-14), 27.4 (C-15), 35.7 (C-16), 43.1 (C-17), 48.4 (C-18), 47.9 (C-19), 151.1 (C-20), 29.7 (C-21), 40.4 (C-22), 28.0 (C-23), 15.5 (C-24), 16.2 (C-25), 16.0 (C-26), 14.6 (C-27), 18.0 (C-28), 109.3 (C-29) and 19.3 (C_{30}).

2.5 Antibacterial Activity

The antibacterial activities of the extracts and the isolated compounds were evaluated against Gram-negative *E. coli* (ATCC 25922) and Gram-positive *S. aureus* (ATCC 25923) using the microtiter broth method [19] with some modifications. All extracts and the isolated compounds were initially adjusted to 2000 μg/mL in 95% ethanol and then serially two-fold diluted with Mueller Hinton Broth (MHB) in microtiter plate. After incubation at 37°C for 24 h, bacterial growth was determined by measuring the absorbance at 600 nm using the labsystems multiskan EX type 335 microplate reader (Helsinki, Finland). Amoxicillin was used as a positive control. The lowest concentration of each sample, which inhibited growth, was taken as the minimal inhibitory concentration (MIC). Triplicate determinations were performed.

2.6 Anticancer Activity

The anticancer activities of the crude extracts and the isolated compounds were determined by the Resazurin Microplate Assay using three human cancer cell lines; KB (oral cancer, ATCC CCL-17), MCF-7 (breast cancer, ATCC HTB-22) and NCI-H 187 (small lung cancer, ATCC CRL-5804) as described by Brien *et al.* [20] with suitable modification. Briefly, the samples were diluted to 50 μg/mL in 0.5% DMSO and then serially three-fold diluted with 0.5% DMSO. Each sample solutions (5 μL) and cell suspension (45 μL) were added to 384-well plates and incubated at 37°C in 5% CO_2 incubator. After 3 days for KB and MCF-7 and 5 days for NCI-H187, 12.5 μL of resazurin solution (62.5 μg/mL) was added to each well, and the plates were then incubated at 37°C for 4 h. The fluorescence signal was measured using a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA). The inhibitory concentration (IC_{50}) represented the concentration that caused 50% reduction in cancer cell line growth. Ellipticine and doxorubicin were used as positive controls and 0.5% DMSO was used as negative control. Triplicate determinations were performed.

2.7 Cytotoxic Activity

The cytotoxicity assay against *Vero* cells (African green monkey kidney, ATCC CCL-81) was determined by the Green Fluorescent Protein (GFP) assay [21]. Each extracts and isolated compounds was first diluted to 50 μg/mL in 0.5% DMSO and then serially three-fold diluted with 0.5% DMSO. The assay was carried out by adding 45 μL of cell suspension at 3.3×10^4 cells/mL to each well of 384-well plates containing 5 μL of each solution and then incubating for 4 days in an incubator at 37°C with 5% CO_2. Fluorescence signals were measured by using SpectraMax M5 microplate reader (Molecular Devices, USA). Ellipticine was used as positive and 0.5% DMSO was used negative controls. The IC_{50} value of each sample has been calculated. Triplicate determinations were performed.

3. RESULTS AND DISCUSSION

3.1 Extraction of Plant Materials

The stems of *M. ferrea* were sequentially extracted with hexane, dichloromethane and methanol. The extracts were evaporated to dryness under reduced pressure to give the crude extracts. The results are shown in Table 1. The methanol extract gave the highest percentage yield, but the hexane extract gave the lowest percentage yield.

3.2 Isolation of the Chemical Constituents of *M. ferrea* Stems

The dichloromethane extract of *M. ferrea* was purified by CC and PTLC on silica gel. The isolated compounds were identified as: friedelin (**1**), the mixture of α-amyrin and β-amyrin (**2**), β-sitosterol (**3**) and lupeol (**4**) (Figure 1). The identifications of all isolated compounds were achieved by spectroscopic analysis on ^{1}H-NMR, ^{13}C-NMR, IR and MS data. These data were also confirmed by comparison with previously reported spectral data.

Compound **1** was obtained as a white solid, mp. 261.5-263.0°C. The EIMS showed molecular ion peak at *m/z* 426, corresponded to the molecular formula of $C_{30}H_{50}O$. The fragment peak at *m/z* 273, together with those at *m/z* 302 and 341, was suggestive of a friedelane derivative with 3-keto substituent [22]. The IR spectrum indicated the presence of carbonyl group from absorption band at ν_{max} 1739 cm^{-1} and the absorption at 2924 and 2852 cm^{-1} are Csp^3-H stretching. The ^{1}H- NMR (400 MHz) spectrum of compound **1** in $CDCl_3$ showed seven methyl groups as singlet at δ 0.72 (H-24), 0.87 (H-25), 1.00 (H-26), 1.05 (H-27), 1.17 (H-28), 0.95 (H-29) and 0.99 (H-30). The secondary methyl was appeared as doublet at δ 0.87 (J = 6.82 Hz, Me-23). The ^{13}C-NMR (100 MHz) spectrum of compound **1** in $CDCl_3$ showed twenty nine signals for thirty carbon atoms due to the disappearance of the carbonyl group at position-3. The structure was confirmed by comparison with the reported ^{1}H-NMR and ^{13}C-NMR spectra data [16] of compound **1** which were corresponding to those described for friedelin.

Compound **2** was obtained as a white solid, mp. 188.0-193.0°C. The EIMS analysis of this compound showed the molecular ion peak at *m/z* 426, corresponded to the molecular formula of $C_{30}H_{50}O$. The GC chromatogram of compound **2** showed peaks at t_R = 82.11 and 80.53 min. The EIMS of peak at t_R = 82.11 showed mass spectrum that matched well with mass spectrum of α-amyrin and peak at t_R = 80.53 showed mass spectrum that matched well with mass spectrum of β-amyrin. The IR spectrum showed absorption band at ν_{max} 3373 cm^{-1}, suggesting the presence of free hydroxyl group. The C-O stretching of alcohol

Table 1. The amount and percentage yields of *M. ferrea* extracts.

Crude extract	Weight of extract (g)	% Yield
Hexane extract	14.56	0.80
Dichloromethane extract	19.11	1.05
Methanol extract	52.96	2.91

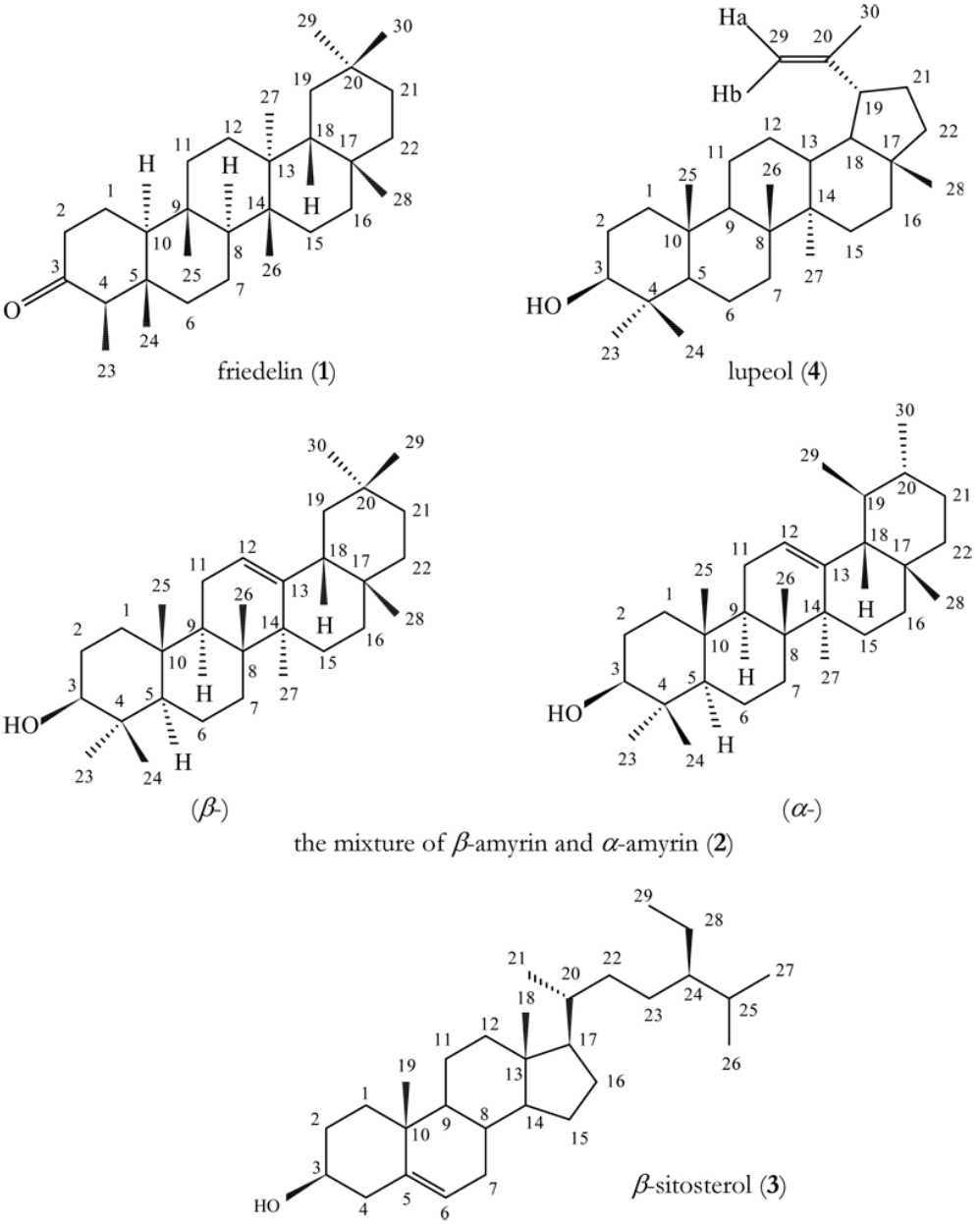

Figure 1. The structures of isolated chemical constituents from the stems of *M. ferrea*.

appeared at ν_{max} 1097 cm^{-1}. The ^{1}H- NMR (400 MHz) spectrum of compounds showed olefinic methine proton of α-amyrin and β-amyrin as triplet (J=3.54 Hz) at δ 5.12 ppm and 5.16 ppm, respectively. The oxymethine proton at position-3 of α-amyrin and β-amyrin as doublet of doublet were appeared at δ 3.21 ppm (J = 5.05, 11.12 Hz) and 3.20 ppm (J = 4.55, 10.86 Hz). The structure was confirmed by comparison with the reported ^{1}H-NMR spectra data [23], IR and MS spectra data of compound **2** was identified as the mixture of α-amyrin and β-amyrin.

Compound **3** was obtaind as a white solid, mp. 138.0-140.0°C. The EIMS analysis of this compounds showed molecular ion peak at *m/z* 414, corresponding to the molecular formula of $C_{29}H_{50}O$. Infrared spectroscopic analysis revealed that the absorption at 3253 cm^{-1}, suggesting the presence of free hydroxyl group. The C-O stretching of alcohol appeared at 1049 cm^{-1}. The C=C stretching appeared at 1662 cm^{-1}. The absorption at 2936 and 2850 cm^{-1} are Csp^3-H stretching. The ^{1}H-NMR (400 MHz) spectrum of compound **3** in $CDCl_3$ showed six methyl groups at d 0.68 (*s*, H-18), 0.83 (*d*, J = 7.32 Hz, H-26), 0.82 (*d*, J = 7.07 Hz, H-27), 0.86 (*t*, J = 7.58 Hz, H-29), 0.93 (*d*, J = 6.57 Hz, H-21) and 1.01 (*s*, H-19) ppm. The doublet signal at d 5.35 ppm (J = 5.24) confirmed the presence of an olefinic methine proton at position-6. The multiplet signal at δ 3.52 was assigned to the proton adjacent the hydroxyl group. The ^{13}C-NMR (100 MHz) spectrum of compound **3** in $CDCl_3$ showed twenty nine signals for twenty nine carbon atoms. The structure was confirmed by comparison with the reported ^{1}H-NMR and ^{13}C-NMR spectra data [17,24] of compound **1** which were corresponding to those described for β-sitosterol.

Compound **4** was obtaind as a white solid, mp. 213.0-214.5°C. The EIMS analysis of this compounds showed molecular ion peak at *m/z* 426, corresponding to the molecular formula of $C_{30}H_{50}O$. The IR spectrum of compound **4** showed absorption band at ν_{max} 3314 cm^{-1} suggesting the presence of free hydroxyl group and at 1454 cm^{-1} for the exomethylene group. The absorption at 2944 and 2852 cm^{-1} are Csp^3-H stretching. The ^{1}H-NMR (400 MHz) spectrum of compound **4** in $CDCl_3$ showed the exomethylene protons of C-29 at δ 4.67 (*brs*, H_a-29) and 4.55 (*brs*, H_b-29). The proton signal at δ 3.18 (*dd*, J = 11.12, 5.24 Hz) was assigned to an oxymethine proton at C-3. The methine proton signal of position-19 appeared at δ 2.37 (*dt*, J = 11.10, 5.70 Hz). The methyl proton signal of C-30 was appeared as singlet at δ 1.68 ppm. The signals of other methyl protons were appeared as singlet at 0.76 (H-24), 0.79 (H-28), 0.83 (H-25), 0.93 (H-27), 0.96 (H-23) and 1.05 (H-26) ppm. The ^{13}C-NMR (100 MHz) spectrum of compound **4** in $CDCl_3$ showed thirty signals for thirty carbon atoms. The exomethylene carbon appeared at 109.3 ppm, the quaternary carbon attached to be exomethylene at 151.1 ppm and the oxygenated methane at 79.2 ppm. The structure was confirmed by comparison with the reported ^{1}H-NMR and ^{13}C-NMR spectra data [18,25] of compound **4** which was elucidated the structure as lupeol.

3.3 Antibacterial Activity

The antibacterial activities of the hexane, dichloromethane and methanol extracts of the *M. ferrea* stems were determined using microtitre broth method. Antibacterial studies were carried out *in vitro* against *E. coli* and *S. aureus*. Gentamicin and amoxicillin were used as positive controls. The results are shown in Table 2.

The methanol extract exhibited the

Table 2. Antibacterial activities of the extracts and the isolated compounds from *M. ferrea* stems.

Samples	MIC (μg/mL)	
	E. coli	*S. aureus*
Hexane extract	500	62.5
Dichloromethane extract	62.5	31.25
Methanol extract	31.25	31.25
Friedelin	250	250
Mixture of α-amyrin and β-amyrin	250	250
Lupeol	250	500
β-sitosterol	1000	1000
Gentamicin	3.13	1.57
Amoxicillin	3.13	3.13

highest antibacterial activity against *E. coli* with the MIC value of 31.25 μg/mL. The methanol extract and the dichloromethane extract exhibited the highest activity against *S. aureus* with the MIC values of 31.25 μg/mL.

All isolated compounds were exhibited moderate antibacterial activity. Friedelin, the mixture of α-amyrin and β-amyrin, lupeol and β-sitosterol showed the antibacterial activity against *E. coli* with the MIC values of 250, 250, 250 and 1000 μg/mL, respectively. They also showed antibacterial activity against *S. aureus* with the MIC values of 250, 250, 500 and 1000 μg/mL, respectively

Some isolated compounds have been reported to exhibit antibacterial activity such as friedelin is found to possess antibacterial activities against *E. coli, S. typhi* and *S. albus* and also antifungal activities against *A. flavus* and *A. niger* [26]. β-Amyrin showed antibacterial activity against *E. coli*, *S. aureus* and *H. pylori* [27]. The mixture of α-amyrin and β-amyrin showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. faecium*, *E. coli*, *S. epidermidis*, *S. saprophyticus, K. neumoniae*, *P. aeruginosa* and *St. maltophilia* [28]. β-Sitosterol is found to be are effective antibacterial and antifungal agent [29]. Lupeol also showed antibacterial activities against *E. coli, B. subtilis* and *S. aureus* [25,30]. Therefore, the antibacterial activity of the extracts and the isolated compounds from this medicinal plant may be used for the development in combating bacterial resistance.

3.4 Anticancer and Cytotoxic Activities

The crude extracts and the isolated compounds were tested for their anticancer and cytotoxic activities. The results are shown in Table 3. The dichloromethane extract exhibited anticancer activities against KB, MCF-7 and NCI-H187 cell lines with the IC_{50} values of 18.01, 28.83 and 18.42 μg/mL, respectively. The methanol extract exhibited anticancer activities against KB and NCI-H187 cell lines with the IC_{50} values of 29.91 and 33.54 μg/mL, respectively.

The mixture of α-amyrin and β-amyrin exhibited anticancer activity against MCF-7 cell line with the IC_{50} value 28.45 μg/mL and lupeol exhibited anticancer activities against KB, MCF-7 and NCI-H187 cell lines with the IC_{50} values of 30.12, 34.25 and 21.56 μg/mL, respectively. All the extracts and the isolated compounds were non cytotoxic to *Vero* cells and these could be good candidates for further study.

Some isolated compounds have been reported to exhibit anticancer activity such as lupeol has been reported to exhibit anticancer property against 451Lu, WM35, B16-F10, B16 2F2, B16-F1, SK-MEL-2, G 361, SK-MEL-2, G 361, SK-MEL-28, MCF-7, K262, CEM, U937, HL60, A2780, Calu-1, A549, As-PC1, MIAPaCa 2, DLD-1, Hela, LNCaP, PC-3, CRW22Rv1, RPMI 8226, Saos 2, SH-10-TC, ACHN, NB-1, HT1080,

Table 3. Anticancer and cytotoxic activities of the extracts and the isolated compounds from *M. ferrea* stems.

Samples	IC_{50} (μg/mL)			
	KB-oral	MCF-7	NCI-H187	*Vero* cell
Hexane extract	NA	NA	NA	NC
Dichloromethane extract	18.01	28.83	18.42	NC
Methanol extract	29.91	NA	33.54	NC
Friedelin	NA	NA	NA	NC
Mixture of α-amyrin and β-amyrin	NA	28.45	NA	NC
Lupeol	30.12	34.25	21.56	NC
β-sitosterol	NA	NA	NA	NC
Ellipticine	0.512	NT	0.875	1.335
Doxorubicin	0.319	0.858	0.050	NT

*NA = no activity, NT = not tested, NC = non-cytotoxic

GOTO, T24 [31] and KB-oral cell line [25]. β-Amyrin has shown anticancer activity in A549 and HL-60 cell lines [23]. The anticancer activity of of *M. ferrea* stems may be synergistic effects of these active compounds. Therefore, *M. ferrea* can play a vital role in development of potent anticancer drug. and offers an option to the pharmaceutical industry of new natural medicine sources.

4. Conclusions

In conclusion, friedelin, the mixture of α-amyrin and β-amyrin, lupeol and β-sitosterol were isolated from the active dichloromethane extract of *M. ferrea* stems. All isolated compounds showed moderately antibacterial activity against *E. coli* and *S. aureus*. The mixture of α-amyrin and β-amyrin exhibited anticancer activity against MCF-7 cell line and lupeol exhibited anticancer activities against KB, MCF-7 and NCI-H187 cell lines. This study is the first report which describes the chemical constituents and bioactive compounds from *M. ferrea* stems. Therefore, according to these results, we suggest that the stems of *M. ferrea* could be another potential source for the new drug development.

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