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Isolation, Structural Elucidation of Alkaloid Constituent and Antimicrobial Activity of Methanol Extract from Berries of Embelia Schimperi

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Abstract
The main target of this project is that to investigate chemical constituent and evaluating antibacterial activities of methanol extract from berries of *Embelia schimperi*. Chromatographic separation of a methanol extract from berries of the plant led to isolation of 2-(pent-4-ol)-N-methyl cyclohexylamine (F_3). The study revealed that the crude extracted berries of the plant have a wide MIC range of 4.22 to 20.14 mg/mL against all tested bacteria strains.
Keywords: *Embelia schimperi*, 2-(pent-4-ol)-N-methyl cyclohexylamine, antimicrobial activity and methanol

Introduction

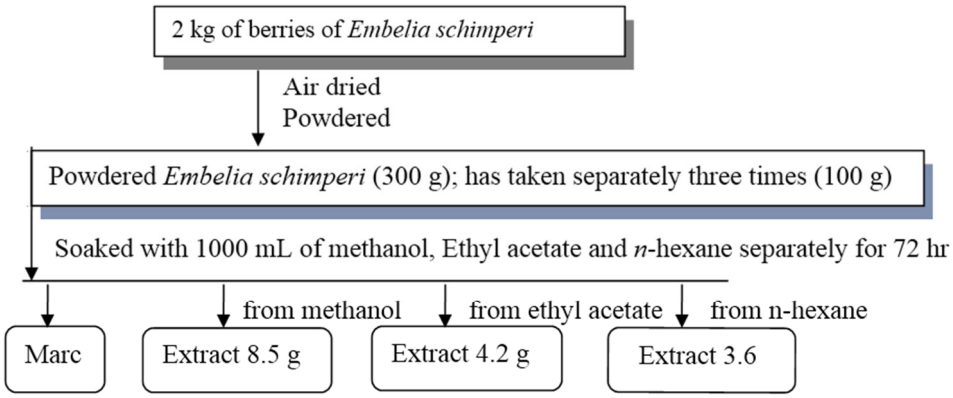
The World Health Organization (WHO) defines traditional medicine as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses and maintain well-being [1]. The extracts of *Embelia schimperi*, *Ocimum gratissimum Plectranthus barbatus*, *Maerua decumbens* and *Conzya floribunda* showed varies degree of antibacterial activity against Gram negative bacterial [2]. Methyleneoxy bridged - oleanane type pentacyclic triterpenoids with antibacterial activity against *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis* were reported from the*E. schimperi* stem bark in Kenya [3] which suggests that antibacterial activity against Gramnegative bacteria reported in this paper might due to the presence of oleanane triterpenes. Another antibacterial investigation conducted by disc diffusion using pure compounds and crude extracts from *E. schimperi* stem ethyl acetate grown in Kenya demostrated that the crude extract was inactive while 2,5-dihydroxy-3- methyl-1,4-benzoquinone showed significant activities against Gram-negative *Salmonella* spp*., Proteus* spp*., P. aeruginosa, K. pneumoniae,* and Gram-positive *E. coli, Shigella dysentriae* and *Staphylococcus aureus* [4]. However another antibacterial investigation from Kenya conducted by using Embelin from *E.schimperi* fruit ethyl acetate extract and Embelin synthetic derivative indicated they were inactive against *P. aeruginosa and E. coli* [5].

Materials and methods

Plant collection and identification

The berries of *Embelia schimperi* were collected from Oromia region, Horo Guduru Wellaga Zone in Horo woreda, Loti-Ano kebele, which is 334 km west of Addis Ababa. The plant was identified by botanists in the Department of Biology, Addis Ababa University.

Extraction and isolation of the compound



Scheme1. General Procedures followed in the extraction of berries of *Embelia schimperi*

By TLC monitoring 7.5 g of the crude extract of methanol was applied on to a column packed with n-hexane silica gel 210 g**.** The column was eluted using solvent system: - pure *n*-hexane, *n*-hexane/EtOAc, pure

chloroform, chloroform/methanol and pure methanol by increasing the volume ratio and generally 41 fractions were collected and further chromatographic purification of the methanol extract takes place from *n*-hexane/EtOAc (9:1) solvent system, fractions 7- 9 was mixed gave compound coded as F_3.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) *Embelia schimperi* methanol extract was evaluated according to method described [2] [6].

Analysis of the isolated compound

NMR spectra were recorded on a Bruker Advance instrument (400 MHz and 100 MHz) and with TMS as an internal standard (chemical shifts in δ, ppm). The isolated compound was dissolved in DMSO-d_6 and analyzed with one-dimensional NMR (proton ^{1}H, carbon ^{13}C) and LC-MS.

Results and discussion

Ground berries part of *Embelia schimperi* (100 g) were subjected to exhaustive extraction with *n*-hexane, ethyl acetate and methanol separately and concentrated under reduced pressure using Rota vapor and yielded n-hexane extract (3.6 g), ethyl acetate extract (4.2 g) and a methanol extract (8.5 g). Based on TLC analysis and yield the methanol extract was selected for further isolation. Chromatographic purification of the methanol extract gave compounds coded F_3 and antimicrobial activity tests were conducted from the crude methanol extract of berries of the plant using standard procedures. The minimum inhibitory concentration (MIC) of *Embelia schimperi,* extract was evaluated against six Gram-negative bacteria has the MIC range against different indicator bacteria was 4.22 to 20.14 mg/mL. This suggests that they have a wider antibacterial spectrum and they can be used for treating more than one infections caused by different Gram-negative bacteria. Disc diffusion method was used and zones of inhibition, after respective incubation periods, were used to quantify antimicrobial activity and results are summarized in Table 1.

Table 1. Antimicrobial activity test results

Solvent extract	Minimum inhibitory concentration MIC(mg/mL)					
	K.pneumoniae	*S.kisarawe*	*P.aerupinosa*	*S.typii*	*P.mirabilis*	*E.coil*
Methanol	9.35	11.32	4.22	7.01	14.5	20.14

Characterization of compound F_3

Compound F_3 was obtained as an amorphous dark brown substance isolated from MeOH extract and the compound was characterized as follows. Its molecular formula, $C_{12}H_{25}ON$ was determined by negative LC-MS and NMR spectra. In the negative LC-MS spectrum, the quasi-molecular ion peak was at m/z 199.31 [M-H]. In the UV spectrum revealed absorption maximum λ_{max} at 216 nm indicating that the molecule has lone pair electron chromophores. In the IR (KBr) spectrum the absorption band at 3435 cm^{-1} due to secondary amine (R_2NH) and hydroxyl group and strong absorption band at 2923 cm^{-1} and medium absorption band at 1465 cm^{-1} due to saturated C-H stretching. ^{1}H-NMR spectrum revealed signals at δ_H 2.72 (1H, m) is due to proton of methine attached to carbon bearing of secondary amine and δ_H 2.18 multiplet integrations for one proton of methine. Signals at δ_H 2.04-1.25 (14H, m) are due to methylene protons. Sharp singlet peak at δ_H 3.18 (3H) N-methyl proton and a signal at δ_H 0.88 (3H, d) is due to methyl protons. ^{13}C-NMR spectrum there was twelve carbon signals. Three tertiary carbons at δ_C 79.61, 49.05 and 29.34 and very intense signals at δ_C 79.61 which represents an sp^3 oxygenated methine bearing hydroxyl group, the signals at the δ_C 49.05 was assigned to the carbons that bearing amine part and δ_C 29.34 also carbons that links cyclohexane bearing hydroxyl group. The signals at δ_C 34.13, 31.74, 29.47, 27.06, 24.94 and 22.53 were assigned to the secondary carbons, two carbon signals at δ_C 29.47 are overlapped, and the carbon signal at the δ_C 40.21 is attributed to methyl linked to nitrogen and δ_C 14.39 stands for aliphatic methyl group. The multiplicity of each carbon atom was determined using DEPT-135 experiment, which revealed the presence of two methyl groups (that is attached to nitrogen and the carbon bearing of alcohol), seven methylene and three methine carbons. (Table 2)

Table 2. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectral data of F_3 in DMSO-d_6

Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)	DEPT-135 (ppm)
1	2.72(1H, dt)	49.05	49.05
2	2.18(1H, m)	29.34	29.47
3	2.04(2H, dt)	31.73	31.74
4, 5	1.95(4H, m)	29.47	29.17
6	2.04(2H, dt)	34.12	34.13
N-CH_3	3.18(3H)	40.21	40.21
1`	1.34(2H, dt)	24.94	24.94
2`	1.25(2H, m)	22.53	22.53
3`	1.48(2H, dt)	27.06	27.06
4`	4.12(1H, m)	79.61	79.61
5`	0.88(3H, d)	14.39	14.39

Based on the NMR (1D), UV, IR and LC-MS spectra (table 2) the structure for the compound F_3 was 2-(pent-4-ol)-N-methyl cyclohexylamine. (Fig 1)

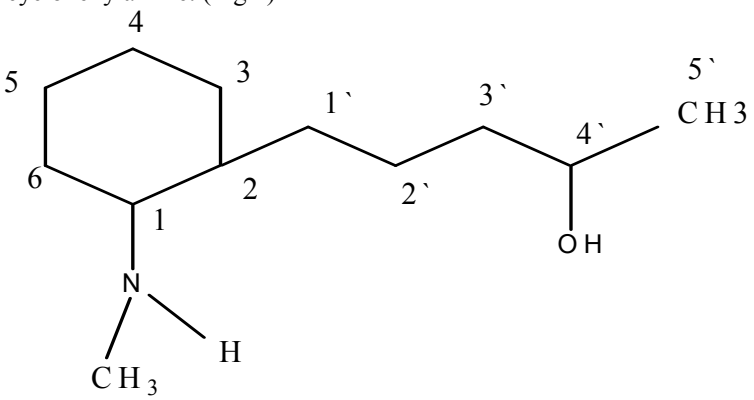


Fig. 1 The structure of compound (F_3)

Conclusions

This work resulted in the isolation of alkaloid compound coded as (F_3) from the berries of the *Embelia schimperi*. The structure of the compound was characterized on the basis of spectral data (UV, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, IR and LC-MS) as well as comparison with the literature data. Methanol extract exhibited a wider antibacterial spectrum and they can be used for treating more than one infections caused by different Gram-negative bacteria.

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